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(71) **Applicant** (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

**(72) Inventors; and**

(75) **Inventors/Applicants (for US only):** FALCO, Saverio, Carl [US/US]; 1902 Millers Road, Arden, DE 19810 (US). ALLEN, Stephen, M. [US/US]; 12 Stanton Avenue, West Chester, PA 19382 (US). RAFALSKI, J., Antoni [US/US]; 2028 Longcome Drive, Wilmington, DE 19810 (US). HITZ, William, D. [US/US]; 404 Hillside Road, Wilmington, DE 19807 (US). KINNEY, Anthony, John [US/US]; 609 Lore Avenue, Wilmington, DE 19809 (US). ABELL, Lynn, Marie [US/US]; 5 Laurel Court, Wilmington, DE 19808 (US). THORPE, Catherine, Jane [GB/GB]; 120 Ross Street, Cambridge CB1 3BU (GB).

(74) Agent: MAJARIAN, William, R.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

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(54) Title: PLANT AMINO ACID BIOSYNTHETIC ENZYMES

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**(57) Abstract**

This invention relates to an isolated nucleic acid fragment encoding a plant enzyme that catalyzes steps in the biosynthesis of lysine, threonine, methionine, cysteine and isoleucine from aspartate, the enzyme a member selected from the group consisting of: dihydroadipylsuccinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the enzyme in a transformed host cell.

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## TITLE

### PLANT AMINO ACID BIOSYNTHETIC ENZYMES

This application claims the benefit of U.S. Provisional Application No. 60/048,771, filed June 6, 1997, and U.S. Provisional Application No. 60/049,443, filed June 12, 1997.

## FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding enzymes involved in amino acid biosynthesis in plants and seeds.

## BACKGROUND OF THE INVENTION

Many vertebrates, including man, lack the ability to manufacture a number of amino acids and therefore require these amino acids preformed in the diet. These are called essential amino acids. Human food and animal feed, derived from many grains, are deficient in essential amino acids, such as lysine, the sulfur amino acids methionine and cysteine, threonine and tryptophan. For example, in corn (*Zea mays L.*) lysine is the most limiting amino acid for the dietary requirements of many animals. Soybean (*Glycine max L.*) meal is used as an additive to corn-based animal feeds primarily as a lysine supplement. Thus, an increase in the lysine content of either corn or soybean would reduce or eliminate the need to supplement mixed grain feeds with lysine produced via fermentation of microbes.

Furthermore, in corn the sulfur amino acids are the third most limiting amino acids, after lysine and tryptophan, for the dietary requirements of many animals. The use of soybean meal, which is rich in lysine and tryptophan, to supplement corn in animal feed is limited by the low sulfur amino acid content of the legume. Thus, an increase in the sulfur amino acid content of either corn or soybean would improve the nutritional quality of the mixtures and reduce the need for further supplementation through addition of more expensive methionine.

Lysine, threonine, methionine, cysteine and isoleucine are amino acids derived from aspartate. Regulation of the biosynthesis of each member of this family is interconnected (see Figure 1). One approach to increasing the nutritional quality of human foods and animal feed is to increase the production and accumulation of specific free amino acids via genetic engineering of this biosynthetic pathway. Alteration of the activity of enzymes in this pathway could lead to altered levels of lysine, threonine, methionine, cysteine and isoleucine. However, few of the genes encoding enzymes that regulate this pathway in plants, especially corn, soybeans and wheat, are available.

The organization of the pathway leading to biosynthesis of lysine, threonine, methionine, cysteine and isoleucine indicates that over-expression or reduction of expression of genes encoding, *inter alia*, threonine synthase, dihydrodipicolinate reductase, diaminopimelate epimerase, threonine deaminase and S-adenosylmethionine synthetase in corn, soybean, wheat and other crop plants could be used to alter levels of these amino acids in human food and animal feed. Accordingly, availability of nucleic acid sequences

encoding all or a portion of these enzymes would facilitate development of nutritionally improved crop plants.

### SUMMARY OF THE INVENTION

The instant invention relates to isolated nucleic acid fragments encoding plant enzymes involved in amino acid biosynthesis. Specifically, this invention concerns isolated nucleic acid fragments encoding the following plant enzymes that catalyze steps in the biosynthesis of lysine, threonine, methionine, cysteine and isoleucine from aspartate: dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase. In addition, this invention relates to nucleic acid fragments that are complementary to nucleic acid fragments encoding the listed plant biosynthetic enzymes.

In another embodiment, the instant invention relates to chimeric genes encoding the amino acid biosynthetic acid enzymes listed above or to chimeric genes that comprise nucleic acid fragments that are complementary to the nucleic acid fragments encoding the enzymes, operably linked to suitable regulatory sequences, wherein expression of the chimeric genes results in production of levels of the encoded enzymes in transformed host cells that are altered (i.e., increased or decreased) from the levels produced in untransformed host cells.

In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene encoding a plant amino acid biosynthetic enzyme operably linked to suitable regulatory sequences, the enzyme selected from the group consisting of: dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase. Expression of the chimeric gene results in production of altered levels of the biosynthetic enzyme in the transformed host cell. The transformed host cells can be of eukaryotic or prokaryotic origin, and include cells derived from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

An additional embodiment of the instant invention concerns a method of altering the level of expression of a plant biosynthetic enzyme in a transformed host cell comprising: a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a plant biosynthetic enzyme selected from the group consisting of dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase, operably linked to suitable regulatory sequences; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of the biosynthetic enzyme in the transformed host cell.

An additional embodiment of the instant invention concerns a method for obtaining a nucleic acid fragment encoding all or substantially all of an amino acid sequence encoding a



plant dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase.

A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of a plant biosynthetic enzyme selected from the group consisting of dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase, the method comprising the steps of: (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a plant biosynthetic enzyme selected from the group consisting of dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase, operably linked to suitable regulatory sequences; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the biosynthetic enzyme in the transformed host cell; (c) optionally purifying the biosynthetic enzyme expressed by the transformed host cell; (d) treating the biosynthetic enzyme with a compound to be tested; and (e) comparing the activity of the biosynthetic enzyme that has been treated with a test compound to the activity of an untreated biosynthetic enzyme, thereby selecting compounds with potential for inhibitory activity.

#### BRIEF DESCRIPTION OF THE

#### DRAWINGS AND SEQUENCE DESCRIPTIONS

The invention can be more fully understood from the following detailed description and the accompanying drawings and sequence descriptions which form a part of this application.

Figure 1 depicts the biosynthetic pathway for the aspartate family of amino acids. The following abbreviations are used: AK = aspartokinase; ASADH = aspartic semialdehyde dehydrogenase; DHDPS = dihydrodipicolinate synthase; DHDPR = dihydrodipicolinate reductase; DAPEP = diaminopimelate epimerase; DAPDC = diaminopimelate decarboxylase; HDH = homoserine dehydrogenase; HK = homoserine kinase; TS = threonine synthase; TD = threonine deaminase; CγS = cystathionine γ-synthase; CβL = cystathionine β-lyase; MS = methionine synthase; CS = cysteine synthase; and SAMS = S-adenosylmethionine synthase.

Figure 2 shows a multiple alignment of the amino acid sequence fragments reported herein encoding dihydrodipicolinate reductase (SEQ ID NOs:2 and 4) and the *Synechocystis* sp. dihydrodipicolinate reductase sequence set forth in DDBJ Accession No. D90899 (SEQ ID NO:5).

Figure 3 shows a multiple alignment of the amino acid sequence fragments reported herein encoding diaminopimelate epimerase (SEQ ID NOs:7, 9, 11, and 13) and the *Synechocystis* sp. diaminopimelate epimerase sequence set forth in DDBJ Accession No. D90917 (SEQ ID NO:14).

Figure 4 shows a multiple alignment of the amino acid sequence fragments reported herein encoding threonine synthase (SEQ ID NOs:16, 18, 20, 22, 24, and 26) and the *Arabidopsis thaliana* threonine synthase sequence set forth in GenBank Accession No. L41666 (SEQ ID NO:27).

5 Figure 5 shows a multiple alignment of the amino acid sequence fragments reported herein encoding threonine deaminase (SEQ ID NOs:29, 31, and 33) to the *Brukholderia capacia* threonine synthase set forth in GenBank Accession No. U40630 (SEQ ID NO:34).

Figure 6 shows the nucleotide sequence alignment of the S-adenosylmethionine synthetase reported herein for corn (SEQ ID NO:35) with the *Oryza sativa*  
 10 S-adenosylmethionine synthetase nucleotide sequence set forth in EMBL Accession No. Z26867 (SEQ ID NO:37).

Figure 7 shows the nucleotide sequence alignment of the S-adenosylmethionine synthetase reported here for soybean (SEQ ID NO:38) with the *Lycopersicon esculentum* S-adenosyl-methionine synthetase nucleotide sequence set forth in EMBL Accession  
 15 No. Z24741 (SEQ ID NO:40).

Figure 8 shows the nucleotide sequence alignment of the S-adenosylmethionine synthetase reported here for wheat (SEQ ID NO:41) with the *Hordeum vulgare* S-adenosylmethionine synthetase nucleotide sequence set forth in DDBJ Accession No. D63835 (SEQ ID NO:43).

20 Amino acid sequence alignments were performed using the Clustal method of alignment (Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153), from the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Nucleotide sequence alignments were a result of the BLASTN search performed with each individual S-adenosylmethionine sequence.

25 The following sequence descriptions and sequence listings attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

SEQ ID NO:1 is the nucleotide sequence comprising the entire cDNA insert in clone csi1n.pk0042.a3 encoding a corn dihydrodipicolinate reductase.

30 SEQ ID NO:2 is the deduced amino acid sequence of a portion of a corn dihydrodipicolinate reductase derived from the nucleotide sequence of SEQ ID NO:1.

SEQ ID NO:3 is the nucleotide sequence comprising a portion of the cDNA insert in clone rls2.pk0017.d3 encoding a rice dihydrodipicolinate reductase.

35 SEQ ID NO:4 is the deduced amino acid sequence of a portion of a rice dihydrodipicolinate reductase derived from the nucleotide sequence of SEQ ID NO:3.

SEQ ID NO:5 is the amino acid sequence of the entire *Synechocystis sp.* dihydrodipicolinate reductase DDBJ Accession No. D90899.

SEQ ID NO:6 is the nucleotide sequence comprising the entire cDNA insert in clone chp2.pk0008.h4 encoding a corn diaminopimelate epimerase.

SEQ ID NO:7 is the deduced amino acid sequence of a portion of a corn diaminopimelate epimerase derived from the nucleotide sequence of SEQ ID NO:6.

SEQ ID NO:8 is the nucleotide sequence comprising a portion of the cDNA insert in clone rls48.pk0036.h10 encoding a rice diaminopimelate epimerase.

5 SEQ ID NO:9 is the deduced amino acid sequence of a portion of a rice diaminopimelate epimerase derived from the nucleotide sequence of SEQ ID NO:8.

SEQ ID NO:10 is the nucleotide sequence comprising a contig formed of portions of sfl1.pk0031.h3, and sgs1c.pk002.k12, and the entire cDNA insert from clones se2.pk0005.f1, and ses8w.pk0010.h11 encoding a soybean diaminopimelate epimerase.

10 SEQ ID NO:11 is the deduced amino acid sequence of a soybean diaminopimelate epimerase derived from the nucleotide sequence of SEQ ID NO:10.

SEQ ID NO:12 is the nucleotide sequence comprising a portion of the cDNA insert in clone wlm24.pk0030.g4 encoding a wheat diaminopimelate epimerase.

15 SEQ ID NO:13 is the deduced amino acid sequence of a portion of a wheat diaminopimelate epimerase derived from the nucleotide sequence of SEQ ID NO:12.

SEQ ID NO:14 is the nucleotide sequence comprising the entire *Synechocystis* sp. diaminopimelate epimerase DDBJ Accession No. D90917.

SEQ ID NO:15 is the nucleotide sequence comprising the entire cDNA insert in clone cc2.pk0031.c9 encoding a corn threonine synthase.

20 SEQ ID NO:16 is the deduced amino acid sequence of a portion of a corn threonine synthase derived from the nucleotide sequence set forth in SEQ ID NO:15.

SEQ ID NO:17 is the nucleotide sequence comprising part of the cDNA insert in clone cs1.pk0058.g5 encoding a corn threonine synthase.

25 SEQ ID NO:18 is the deduced amino acid sequence of a portion of a corn threonine synthase derived from the nucleotide sequence of SEQ ID NO:17.

SEQ ID NO:19 is the nucleotide sequence comprising part of the cDNA insert in clone rls72.pk0018.e7 encoding a rice threonine synthase.

SEQ ID NO:20 is deduced amino acid sequence of a portion of a rice threonine synthase derived from the nucleotide sequence set forth in SEQ ID NO:19.

30 SEQ ID NO:21 is the nucleotide sequence comprising part of the cDNA insert in clone se1.06a03 encoding a soybean threonine synthase.

SEQ ID NO:22 is the deduced amino acid sequence of a portion of a soybean threonine synthase derived from the nucleotide sequence set forth in SEQ ID NO:21.

35 sr1.pk0003.f6 encoding a soybean threonine synthase.

SEQ ID NO:24 is the deduced amino acid sequence of a portion of a soybean threonine synthase derived from the nucleotide sequence set forth in SEQ ID NO:23.

SEQ ID NO:25 is the nucleotide sequence comprising part of the cDNA insert in clone wr1.pk0085.h2 encoding a wheat threonine synthase.

SEQ ID NO:26 is the deduced amino acid sequence of a portion of a wheat threonine synthase derived from the nucleotide sequence set forth in SEQ ID NO:25.

SEQ ID NO:27 is the entire amino acid sequence of an *Arabidopsis thaliana* threonine synthase found in GenBank Accession No. L41666.

5 SEQ ID NO:28 is the nucleotide sequence comprising the entire cDNA insert in clone cen1.pk0064.f4 encoding a corn threonine deaminase.

SEQ ID NO:29 is the deduced amino acid sequence of a portion of a corn threonine deaminase derived from the nucleotide sequence set forth in SEQ ID NO:28.

10 SEQ ID NO:30 is the nucleotide sequence comprising a portion of the cDNA insert in clone sfl1.pk0055.h7 encoding a soybean threonine deaminase.

SEQ ID NO:31 is the deduced amino acid sequence of a portion of a soybean threonine deaminase derived from the nucleotide sequence set forth in SEQ ID NO:30.

SEQ ID NO:32 is the nucleotide sequence comprising the entire cDNA insert in clone sre.pk0044.f3 encoding a soybean threonine deaminase.

15 SEQ ID NO:33 is the deduced amino acid sequence of a portion of a soybean threonine deaminase derived from the nucleotide sequence set forth in SEQ ID NO:32.

SEQ ID NO:34 is the entire amino acid sequence of a *Burkholderia capacia* threonine deaminase found in GenBank Accession No. U49630.

20 SEQ ID NO:35 is the nucleotide sequence comprising the entire cDNA insert in clone cc3.mn0002.d2 encoding the entire corn S-adenosylmethionine synthetase.

SEQ ID NO:36 is the deduced amino acid sequence of a corn S-adenosylmethionine synthetase derived from the nucleotide sequence set forth in SEQ ID NO:35.

SEQ ID NO:37 is the entire nucleotide sequence of a *Oryza sativa* S-adenosyl-methionine synthetase found in EMBL Accession No. Z26867.

25 SEQ ID NO:38 is the nucleotide sequence of the entire cDNA insert in clone s2.12b06 encoding the entire soybean S-adenosyl-methionine synthetase.

SEQ ID NO:39 is the deduced amino acid sequence of the entire soybean S-adenosyl-methionine synthetase derived from the nucleotide sequence set forth in SEQ ID NO:38.

30 SEQ ID NO:40 is the entire nucleotide sequence of a *Lycopersicon esculentum* S-adenosyl-methionine synthetase found in EMBL Accession No. Z24741.

SEQ ID NO:41 is the nucleotide sequence comprising a contig formed of portions of the cDNA inserts in clones wre1.pk0002.c12, wle1n.pk0070.b8, wkm1c.pk0003.g4, wlk1.pk0028.d3, wre1n.pk170.d8, wr1.pk0086.d5, wr1.pk0103.h8, and wre1n.pk0082.b2 encoding a portion of a wheat S-adenosyl-methionine synthetase.

35 SEQ ID NO:42 is the deduced amino acid sequence of a wheat S-adenosyl-methionine synthetase derived from the nucleotide sequence set forth in SEQ ID NO:41.

SEQ ID NO:43 is the entire nucleotide sequence of a *Hordeum vulgare* S-adenosyl-methionine synthetase found in DDBJ Accession No. D63835.

The Sequence Descriptions contain the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IYUB standards described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

#### DETAILED DESCRIPTION OF THE INVENTION

In the context of this disclosure, a number of terms shall be utilized. As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA. As used herein, "contig" refers to an assemblage of overlapping nucleic acid sequences to form one contiguous nucleotide sequence. For example, several DNA sequences can be compared and aligned to identify common or overlapping regions. The individual sequences can then be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate alteration of gene expression by antisense or co-suppression technology or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary sequences.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% identity with the gene to be suppressed. Moreover, alterations in a gene which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded protein, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to

produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the protein molecule would also not be expected to alter the activity of the protein. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Moreover, the skilled artisan recognizes that substantially similar sequences encompassed by this invention are also defined by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C), with the sequences exemplified herein. Preferred substantially similar nucleic acid fragments of the instant invention are those nucleic acid fragments whose DNA sequences are 80% identical to the DNA sequence of the nucleic acid fragments reported herein. More preferred nucleic acid fragments are 90% identical to the identical to the DNA sequence of the nucleic acid fragments reported herein. Most preferred are nucleic acid fragments that are 95% identical to the DNA sequence of the nucleic acid fragments reported herein. The Clustal multiple alignment algorithm (Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153) was used here with a GAP PENALTY of 10 and a GAP LENGTH PENALTY of 10.

A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to afford putative identification of that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches partial or complete amino acid and nucleotide sequences encoding one or more particular plant proteins. The skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

“Codon degeneracy” refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment that encodes all or a substantial portion of the amino acid sequence encoding the amino acid biosynthetic enzymes as set forth in SEQ ID NOs:2, 4, 7, 9, 11, 13, 16, 18, 20, 22, 24, 26, 29, 31, and 33. The skilled artisan is well aware of the “codon-bias” exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a gene for improved expression in a host cell, it is desirable to design the gene such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

“Synthetic genes” can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form gene segments which are then enzymatically assembled to construct the entire gene. “Chemically synthesized”, as related to a sequence of DNA, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of DNA may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. “Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign” gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

“Coding sequence” refers to a DNA sequence that codes for a specific amino acid sequence. “Regulatory sequences” refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the

associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

“Promoter” refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an “enhancer” is a DNA sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters which cause a gene to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”. New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg, (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

The “translation leader sequence” refers to a DNA sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency. Examples of translation leader sequences have been described (Turner, R. and Foster, G.D. (1995) *Molecular Biotechnology* 3:225).

The “3' non-coding sequences” refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht et al., (1989) *Plant Cell* 1:671-680.

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is without introns and that can be translated into protein by the cell. “cDNA” refers to a double-stranded DNA that is complementary to and derived from mRNA. “Sense” RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the



cell. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (U.S. Pat. No. 5,107,065). The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated yet has an effect on cellular processes.

The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. "Antisense inhibition" refers to the production of antisense RNA transcripts capable of suppressing the expression of the target protein. "Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms. "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020).

"Altered levels" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature" protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the secretory system (Chrispeels, J.J., (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include Agrobacterium-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Pat. No. 4,945,050).

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

Nucleic acid fragments encoding at least a portion of several plant amino acid biosynthetic enzymes have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences using the BLAST algorithms well known to those skilled in the art. Table 1 lists the amino acid biosynthetic enzymes that are described herein, and the designation of the cDNA clones that comprise the nucleic acid fragments encoding these enzymes.

**TABLE 1**  
**Amino Acid Biosynthetic Enzymes**

Enzyme	Clone	Plant
dihydrodipicolinate reductase	cs1.pk0083.b10	corn
	rls2.pk0017.d3	rice
diaminopimelate epimerase	chp2.pk0008.h4	corn
	rls48.pk0036.h10	rice
	se2.pk0005.f1	soybean
	ses8w.pk0010.f11	soybean
	sfl1.pk0031.h3	soybean
	sgs1c.pk002.k12	soybean
	wlm24.pk0030.g4	wheat
threonine synthase	cc2.pk0031.c9	corn
	cs1.pk0058.g5	corn
	rls72.pk0018.e7	rice
	se1.06a03	soybean
	srl.pk0003.f6	soybean
	wrl.pk0085.h2	wheat
threonine deaminase	cen1.pk0064.f4	corn
	sfl1.pk0055.h7	soybean
	sre.pk0044.f3	soybean

Enzyme	Clone	Plant
s-adenosylmethionine synthase	cc3.mn0002.d2	corn
	se2.12b06	soybean
	wre1.pk0002.c12	wheat
	wle1n.pk0070.b8	wheat
	wkmlc.pk0003.g4	wheat
	wlk1.pk0028.d3	wheat
	wre1n.pk170.d8	wheat
	wr1.pk0086.d5	wheat
	wr1.pk0103.h8	wheat
	wre1n.pk0082.b2	wheat

The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous enzymes from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other amino acid biosynthetic enzymes, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be designed and synthesized by methods known in the art (Maniatis). Moreover, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primers DNA labeling, nick translation, or end-labeling techniques. or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part of or full-length of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow

the RACE protocol (Frohman et al., (1988) *PNAS USA* 85:8998) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al., (1989) *PNAS USA* 86:5673; Loh et al., (1989) *Science* 243:217). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman, M.A. and Martin, G.R., (1989) *Techniques* 1:165).

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner, R.A. (1984) *Adv. Immunol.* 36:1; Maniatis).

The nucleic acid fragments of the instant invention may be used to create transgenic plants in which the disclosed biosynthetic enzymes are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of free amino acids in those cells.

Overexpression of the biosynthetic enzymes of the instant invention may be accomplished by first constructing chimeric genes in which the coding region are operably linked to promoters capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric genes may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric genes may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant chimeric genes can then be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the plasmid vector in order to successfully transform, select and propagate host cells containing the chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in different levels and patterns of expression (Jones et al., (1985) *EMBO J.* 4:2411-2418; De Almeida et al., (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant biosynthetic enzymes to different cellular compartments, or to facilitate their secretion from the cell. It is thus envisioned that the chimeric genes described above may be further supplemented by altering the coding sequences to encode enzymes with appropriate intracellular targeting sequences such as transit sequences (Keegstra, K. (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels, J.J., (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel, N. (1992) *Plant Phys.* 100:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

It may also be desirable to reduce or eliminate expression of the genes encoding the instant biosynthetic enzymes in plants for some applications. In order to accomplish this, chimeric genes designed for co-suppression of the instant biosynthetic enzymes can be constructed by linking the genes or gene fragments encoding the enzymes to plant promoter sequences. Alternatively, chimeric genes designed to express antisense RNA for all or part of the instant nucleic acid fragments can be constructed by linking the genes or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

The instant amino acid biosynthetic enzymes (or portions of the enzymes) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to the enzymes by methods well known to those skilled in the art. The antibodies are useful for detecting the enzymes *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant amino acid biosynthetic enzymes are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct chimeric genes for production of the instant amino acid biosynthetic enzymes. These chimeric genes could then be introduced into appropriate microorganisms via transformation to provide high level expression of the enzymes. An example of a vector for high level expression of the instant amino acid biosynthetic enzymes in a bacterial host is provided (Example 11).

Additionally, the instant plant amino acid biosynthetic enzymes can be used as a targets to facilitate design and/or identification of inhibitors of the enzymes that may be useful as herbicides. This is desirable because the enzymes described herein catalyze various steps in a pathway leading to production of several essential amino acids. Accordingly, inhibition of the activity of one or more of the enzymes described herein could lead to inhibition of amino acid biosynthesis sufficient to inhibit plant growth. Thus, the instant plant amino acid biosynthetic enzymes could be appropriate for new herbicide discovery and design.

All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al., (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein, D. et al., (1980) *Am. J. Hum. Genet.* 32:314-331).

The production and use of plant gene-derived probes for use in genetic mapping is described in R. Bernatzky, R. and Tanksley, S. D. (1986) *Plant Mol. Biol. Reporter* 4(1):37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel, J. D., et al., In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic press 1996, pp. 319-346, and references cited therein).

In another embodiment, nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask, B. J. (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several to several hundred KB; see Laan, M. et al. (1995) *Genome Research* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian, H. H. (1989) *J. Lab. Clin. Med.* 114(2):95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield, V. C. et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren, U. et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov, B. P. (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter, M. A. et al. (1997) *Nature Genetics* 7:22-28) and Happy Mapping

(Dear, P. H. and Cook, P. R. (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer, (1989) *Proc. Natl. Acad. Sci USA* 86:9402; Koes et al., (1995) *Proc. Natl. Acad. Sci USA* 92:8149; Bensen et al., (1995) *Plant Cell* 7:75). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase or S-adenosylmethionine synthetase. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding a dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase or S-adenosylmethionine synthetase can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase gene product.

#### EXAMPLES

The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLE 1Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

5

TABLE 2cDNA Libraries from Corn and Soybean Tissues

Library	Tissue	Clone
cc2	Corn Callus, Partially Differentiated, 2 Weeks After Subculture	cc2.pk0031.c9
cc3	Corn Callus, Mature Somatic Embryo	cc3.mn0002.d2
cen1	Corn Endosperm 12 Days After Pollination	cen1.pk0064.f4
chp2	Corn Leaf, 11 Day Old Plant	chp2.pk0008.h4
cs1	Corn Leaf, Sheath 5 Week Old Plant	cs1.pk0058.g5
csi1n	Corn Silk*	csi1n.pk0042.a3
rls2	Rice Leaf 15 Days After Germination, 2 Hours After Infection of Strain Magaporthe grisea 4360-R-67 (AVR2-YAMO); Susceptible	rls2.pk0017.d3
rls48	Rice Leaf 15 Days After Germination, 48 Hours After Infection of Strain Magaporthe grisea 4360-R-67 (AVR2-YAMO); Susceptible	rls48.pk0036.h10
rls72	Rice Leaf 15 Days After Germination, 72 Hours After Infection of Strain Magaporthe grisea 4360-R-67 (AVR2-YAMO); Susceptible	rls72.pk0018.e7
s2	Soybean Seed, 19 Days After Flowering	s2.12b06
se1	Soybean Embryo 7 Days After Flowering	se1.06a03
se2	Soybean Embryo 10 Days After Flowering	se2.pk0005.f1
ses8w	Mature Soybean Embryo 8 Weeks After Subculture	ses8w.pk0010.h11
sf11	Soybean Immature Flower	sf11.pk0055.h7 sf11.pk0031.h3
sgs1c	Soybean Seeds 4 Hours After Germination	sgs1c.pk002.k12
sr1	Soybean Root From 10 Day Old Seedlings	sr1.pk0003.f6
sre	Soybean Root Elongation 4-5 Days After Germination	sre.pk0044.f3
wkm1c	Wheat Kernel Malted 55 Hours at 22 Degrees Celsius	wkm1c.pk0003.g4
wle1n	Wheat Leaf From 7 Day Old Etiolated Seedling*	wle1n.pk0070.b8
wlk1	Wheat Seedlings 1 Hour After Treatment with Fungicide**	wlk1.pk0028.d3
wlm24	Wheat Seedlings 24 Hours After Inoculation With Erysiphe graminis f. sp tritici	wlm24.pk0030.g4
wr1	Wheat Root From 7 Day Old Seedling	wr1.pk0085.h2 wr1.pk0086.d5 wr1.pk0103.h8
wre1	Wheat Root From 7 Day Old Etiolated Seedling	wre1.pk0002.c12
wre1n	Wheat Root From 7 Day Old Etiolated Seedling*	wre1n.pk0082.b2 wre1n.pk170.d8

\*These libraries were normalized essentially as described in U.S. Pat. No. 5,482,845



\*\*Application of 6-iodo-2-propoxy-3-propyl-4(3*H*)-quinazolinone; synthesis and methods of using this compound are described in USSN 08/545,827, incorporated herein by reference.

5 cDNA libraries were prepared in Uni-ZAP™ XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA). Conversion of the Uni-ZAP™ XR libraries into plasmid libraries was accomplished according to the protocol provided by Stratagene. Upon conversion, cDNA inserts were contained in the plasmid vector pBluescript. cDNA inserts from randomly picked bacterial colonies containing  
10 recombinant pBluescript plasmids were amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences, or plasmid DNA was prepared from cultured bacterial cells. Amplified insert DNAs or plasmid DNAs were sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams, M. D. et al., (1991) *Science* 252:1651).  
15 The resulting ESTs were analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

#### EXAMPLE 2

##### Identification and Characterization of cDNA Clones

ESTs encoding plant amino acid biosynthetic enzymes were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.*  
20 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity  
25 to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) *Nature Genetics* 3:266-272) provided by the  
30 NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

35

#### EXAMPLE 3

##### Characterization of cDNA Clones Encoding Polypeptides Homologous to

##### Dihydrodipicolinate Reductase

The BLASTX search using the nucleotide sequences from clones csi1n.pk0042.a3 and rls2.pk0017.d3 revealed similarity of the protein encoded by the cDNA to *Synechocystis* sp.

dihydrodipicolinate reductase enzyme (DDBJ Accession No. D90899). BLAST pLog values were 12.60 and 11.68 for csi1n.pk0042.a3 and rls2.pk0017.d3, respectively.

The sequence of the entire cDNA insert in clone csi1n.pk0042.a3 was determined and is shown in SEQ ID NO:1; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:2. The amino acid sequence set forth in SEQ ID NO:2 was evaluated by BLASTP, yielding a pLog value of 36.72 versus the *Synechocystis sp.* dihydrodipicolinate reductase sequence. The sequence of a portion of the cDNA insert from clone rls2.pk0017.d3 is shown in SEQ ID NO:3; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:4. Figure 2 presents an alignment of the amino acid sequences set forth in SEQ ID NO:2 and the *Synechocystis sp.* dihydrodipicolinate reductase sequence (SEQ ID NO:5). SEQ ID NO:2 is 40% identical to the *Synechocystis sp.* dihydrodipicolinate reductase sequence (SEQ ID NO:5). Sequence alignments were performed by the Clustal method of alignment (Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153), using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Sequence percent identity calculations were performed by the Jotun Hein method (Hein, J. J. (1990) *Meth. Enz.* 183:626-645) using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI).

Sequence alignments and BLAST scores and probabilities indicate that the instant nucleic acid fragments encode a nearly entire corn dihydrodipicolinate reductase, and a portion of a rice dihydrodipicolinate reductase. These sequences represent the first plant sequences encoding dihydrodipicolinate reductase.

#### EXAMPLE 4

##### Characterization of cDNA Clones Encoding Diaminopimelate Epimerase

The BLASTX search using the nucleotide sequences from clones chp2.pk0008.h4, rls48.pk0036.h10, wlm24.pk0030.g4, and the contig sequences assembled from clones se2.pk0005.fl, ses8w.pk0010.h11, sfl1.pk0031.h3, and sgs1c.pk002.k12 revealed similarity of the proteins encoded by the cDNAs to diaminopimelate epimerase from *Synechocystis sp.* (DDBJ Accession No. D90917). The BLAST results for each of these ESTs are shown in Table 3:

TABLE 3

BLAST Results for Clones Encoding Polypeptides Homologous  
to Diaminopimelate Epimerase

Clone	BLAST pLog Score DDBJ Accession No. D90917
chp2.pk0008.h4	59.16
rls48.pk0036.h10	40.82
The contig of:	98.30
se2.pk0005.f1	
ses8w.pk0010.h11	
sfl1.pk0031.h3	
sgs1c.pk002.k12	
wlm24.pk0030.g4	23.46

- 5       The sequence of the entire cDNA insert in clone chp2.pk0008.h4 was determined and is shown in SEQ ID NO:6; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:7. The amino acid sequence set forth in SEQ ID NO:7 was evaluated by BLASTP, yielding a pLog value of 75.66 versus the *Synechocystis sp.* sequence. The sequence of a portion of the cDNA insert from clone rls48.pk0036.h10 is shown in SEQ ID NO:8; the
- 10       deduced amino acid sequence of this cDNA is shown in SEQ ID NO:9. The nucleotide sequence of the contig assembled from clones se2.pk0005.f1, ses8w.pk0010.h11, sfl1.pk0031.h3, and sgs1c.pk002.k12 was determined and is shown in SEQ ID NO:10; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:11. The amino acid sequence set forth in SEQ ID NO:11 was evaluated by BLASTP, yielding a pLog value of
- 15       98.57 versus the *Synechocystis sp.* sequence. The sequence of a portion of the cDNA insert from clone wlm24.pk0030.g4 is shown in SEQ ID NO:12; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:13. Figure 3 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:7, 9, 11, and 13 and the *Synechocystis sp.* sequence (SEQ ID NO:14). The data in Table 4 represents a calculation of the percent identity of the
- 20       amino acid sequences set forth in SEQ ID NOs: 7, 9, 11, and 13 and the *Synechocystis sp.* sequence.

TABLE 4

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of  
cDNA Clones Encoding Polypeptides Homologous  
to Diaminopimelate Epimerase

Clone	SEQ ID NO.	Percent Identity to DDBJ Accession No. D90917 (SEQ ID NO:16)
chp2.pk0008.h4	7	59
rls48.pk0036.h10	9	74
Contig of:	11	72
se2.pk0005.f1		
ses8w.pk0010.h11		
sfl1.pk0031.h3		
sgs1c.pk002.k12		
wlm24.pk0030.g4	13	65

5 Sequence alignments were performed by the Clustal method of alignment (Higgins,  
D.G. and Sharp, P.M. (1989) CABIOS 5:151-153), using the Megalign program of the  
LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Sequence  
percent identity calculations were performed by the Jotun Hein method (Hein. J. J. (1990)  
10 Meth. Enz. 183:626-645) using the Megalign program of the LASARGENE bioinformatics  
computing suite (DNASTAR Inc., Madison, WI).

Sequence alignments and BLAST scores and probabilities indicate that the instant  
nucleic acid fragments encode a nearly entire corn diaminopimelate epimerase  
(chp2.pk0008.h4), a portion of a rice diaminopimelate epimerase (rls48.pk0036.h10), and an  
15 entire soybean diaminopimelate epimerase (se2.pk0005.f1, ses8w.pk0010.h11,  
sfl1.pk0031.h3, and sgs1c.pk002.k12), and a portion of a wheat diaminopimelate epimerase  
(wlm24.pk0030.g4). These sequences represent the first plant sequences encoding  
diaminopimelate epimerase enzyme.

EXAMPLE 520 Characterization of cDNA Clones Encoding Threonine Synthase

The BLASTX search using the EST sequences from clones cc2.pk0031.c9,  
cs1.pk0058.g5, rls72.pk0018.e7, se1.06a03, sr1.pk0003.f6, and wr1.pk0085.h2 revealed  
similarity of the proteins encoded by the cDNAs to threonine synthase from *Arabidopsis*  
*thaliana* (GenBank Accession No. L41666). The BLAST results for each of these ESTs are  
25 shown in Table 5:

TABLE 5

BLAST Results for Clones Encoding Polypeptides Homologous  
to Threonine Synthase

Clone	BLAST pLog Score L41666
cc2.pk0031.c9	56.19
cs1.pk0058.g5	8.00
rls72.pk0018.e7	29.47
se1.06a03	34.15
sr1.pk0003.f6	21.13
wr1.pk0085.h2	29.47

- 5 The sequence of the entire cDNA insert in clone cc2.pk0031.c9 was determined and is shown in SEQ ID NO:15; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:16. The amino acid sequence set forth in SEQ ID NO:16 was evaluated by BLASTP, yielding a pLog value of 166.11 versus the *Arabidopsis thaliana* sequence. BLASTN
- 10 against dbest indicated identity of nucleotides 520 through 684 from cc2.pk0031.c9 with nucleotides 1 through 162 of a corn EST (GenBank Accession No. T18847). The sequence of a portion of the cDNA insert from clone cs1.pk0058.g5 is shown in SEQ ID NO:17; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:18. The sequence of a portion of the cDNA insert from clone rls72.pk0018.e7 is shown in SEQ ID NO:19; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:20. The sequence of a
- 15 portion of the cDNA insert from clone se1.06a03 is shown in SEQ ID NO:21; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:22. The sequence of the entire cDNA insert in clone sr1.pk0003.f6 was determined and is shown in SEQ ID NO:23; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:24. The amino acid sequence set forth in SEQ ID NO:24 was evaluated by BLASTP, yielding a pLog value of
- 20 275.06 versus the *Arabidopsis thaliana* sequence. The sequence of a portion of the cDNA insert from clone wr1.pk0085.h2 is shown in SEQ ID NO:25; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:26. Figure 4 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:16, 18, 20, 22, 24, and 26 and the
- 25 *Arabidopsis thaliana* sequence. The data in Table 6 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:16, 18, 20, 22, 24, and 26 and the *Arabidopsis thaliana* sequence (SEQ ID NO:27).

TABLE 6

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Threonine Synthase

Clone	SEQ ID NO.	Percent Identity to L41666 (SEQ ID NO:29)
cc2.pk0031.c9	16	81.0
cs1.pk0058.g5	18	81.0
rls72.pk0018.e7	20	55.3
se1.06a03	22	80.0
sr1.pk0003.f6	24	84.4
wr1.pk0085.h2	26	50.4

5

Sequence alignments were performed by the Clustal method of alignment (Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153), using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Sequence percent identity calculations were performed by the Jotun Hein method (Hein, J. J. (1990) *Meth. Enz.* 183:626-645) using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI).

Sequence alignments and BLAST scores and probabilities indicate that the instant nucleic acid fragments encode portions of a corn threonine synthase (cc2.pk0031.c9 and cs1.pk0058.g5), a portion of a rice threonine synthase (rls72.pk0018.e7), portions of a soybean threonine synthase (se1.06a03 and sr1.pk0003.f6), and a portion of a wheat threonine synthase (wr1.pk0085.h2). These sequences represent the first corn, rice, soybean, and wheat sequences encoding threonine synthase.

#### EXAMPLE 6

##### Characterization of cDNA Clones Encoding Threonine Deaminase

The BLASTX search using the EST sequence from clone cen1.pk0064.f4 revealed similarity of the protein encoded by the cDNA to threonine deaminase from *Brukholderia capacia* (GenBank Accession No. U40630; pLog = 31.38). The BLASTX search using the EST sequences from clones sfl1.pk0055.h7 and sre.pk0044.f3 revealed similarity of the proteins encoded by the cDNAs to threonine deaminase from *Solanum tuberosum* and *Brukholderia capacia* (EMBL Accession No. X67846 and GenBank Accession No. U40630, respectively). BLAST pLog values were 36.55 and 31.79 for sfl1.pk0055.h7, and 19.47 and 14.51 for sre.pk0044.f3.

The sequence of the entire cDNA insert in clone cen1.pk0064.f4 was determined and is shown in SEQ ID NO:28; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:29. The amino acid sequence set forth in SEQ ID NO:29 was evaluated by BLASTP, yielding a pLog value of 134.85 versus the *Brukholderia capacia* sequence. The sequence of a portion of the cDNA insert from clone sfl1.pk0055.h7 is shown in SEQ ID

NO:30; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:31. The sequence of the entire cDNA insert in clone sre.pk0044.f3 was determined and is shown in SEQ ID NO:32; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:33. The amino acid sequence set forth in SEQ ID NO:33 was evaluated by BLASTP, yielding pLog values of 19.24 versus the *Solanum tuberosum* sequence and 15.19 versus the *Brukholderia capacia* threonine deaminase sequence. Figure 5 presents an alignment of the amino acid sequences set forth in SEQ ID NOs:29, 31, and 33 and the *Brukholderia capacia* (SEQ IDNO:34) sequence. The data in Table 7 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs:29, 31, and 33 and the *Brukholderia capacia* sequence.

TABLE 7

Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to Threonine Deaminase

Clone	SEQ ID NO.	Percent Identity to U40630 (SEQ ID NO:36)
cen1.pk0064.f4	29	61.0
sfl1.pk0055.h7	31	47.9
sre.pk0044.f3	33	46.0

Sequence alignments were performed by the Clustal method of alignment (Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153), using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Sequence percent identity calculations were performed by the Jotun Hein method (Hein, J. J. (1990) *Meth. Enz.* 183:626-645) using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI)

Sequence alignments and BLAST scores and probabilities indicate that the instant nucleic acid fragments encode a nearly entire corn threonine deaminase (cen1.pk0064.f4) and portions of a soybean threonine deaminase (sfl1.pk0055.h7 and sre.pk0044.f3). These sequences represent the first corn and soybean sequences encoding threonine deaminase.

#### EXAMPLE 7

##### Characterization of cDNA Clones Encoding S-adenosylmethionine synthetase

The BLASTX search using the nucleotide sequence from clone cc3.mn0002.d2 revealed similarity of the protein encoded by the cDNA to S-adenosylmethionine synthetase from *Oryza sativa* (EMBL Accession No. Z26867; pLog = 99.03). The sequence of the entire cDNA insert in clone cc3.mn0002.d2 was determined and is shown in SEQ ID NO:35; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:36. The nucleotide sequence set forth in SEQ ID NO:35 was evaluated by BLASTN, yielding a pLog value larger than 200 versus the *Oryza sativa* sequence. Figure 6 presents an alignment of the

nucleotide sequences set forth in SEQ ID NO:35 and the *Oryza sativa* sequence (SEQ ID NO:37). The nucleotide sequence in SEQ ID NO:35 is 88% identical over 1216 nucleotides to the nucleotide sequence of the *Oryza sativa* S-adenosylmethionine synthetase.

5 The BLASTX search using the nucleotide sequence from clone s2.12b06 revealed similarity of the protein encoded by the cDNA to S-adenosylmethionine synthetase from *Lycopersicon esculentum* (EMBL Accession No. Z24741; pLog = 62.62). The sequence of the entire cDNA insert in clone s2.12b06 was determined and is shown in SEQ ID NO:38; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:39. The nucleotide sequence set forth in SEQ ID NO:38 was evaluated by BLASTN, yielding a pLog value  
10 larger than 200 versus the *Lycopersicon esculentum* sequence. Figure 7 presents an alignment of the nucleotide sequences set forth in SEQ ID NO:38 and the *Lycopersicon esculentum* sequence (SEQ ID NO:40). The nucleotide sequence set forth in SEQ ID NO:38 is 82 % identical over 1210 nucleotides to the *Lycopersicon esculentum* sequence.

The BLASTX search using the nucleotide sequence from the contig assembled from  
15 clones wre1.pk0002.c12, wle1n.pk0070.b8, wkm1c.pk0003.g4, wlk1.pk0028.d3, wre1n.pk170.d8, wr1.pk0086.d5, wr1.pk0103.h8, and wre1n.pk0082.b2 revealed similarity of the protein encoded by the contig to S-adenosylmethionine synthetase from *Hordeum vulgare* (DDBJ Accession No. D63835) with a pLog value larger than 200. The nucleotide sequence of the contig assembled from clones wre1.pk0002.c12, wle1n.pk0070.b8,  
20 wkm1c.pk0003.g4, wlk1.pk0028.d3, wre1n.pk170.d8, wr1.pk0086.d5, wr1.pk0103.h8, and wre1n.pk0082.b2 is shown in SEQ ID NO:41; the deduced amino acid sequence of this cDNA is shown in SEQ ID NO:42. Figure 8 presents an alignment of the nucleotide sequence set forth in SEQ ID NO:41 and the *Hordeum vulgare* sequence (SEQ ID NO:43). The SEQ ID NO:41 is 92% identical to the *Hordeum vulgare* sequence.

25 Sequence alignments and BLAST scores and probabilities indicate that the instant nucleic acid fragments encode entire or nearly entire corn, soybean, or wheat S-adenosylmethionine synthetase. These sequences represent the first corn, soybean, or wheat sequences encoding S-adenosylmethionine synthetase.

#### EXAMPLE 8

#### 30 Expression of Chimeric Genes in Monocot Cells

A chimeric gene comprising a cDNA encoding an amino acid biosynthetic enzyme in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain  
35 reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers and under appropriate experimental conditions. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. The amplified DNA can then be digested with restriction enzymes NcoI and SmaI and fractionated on a 0.7% low melting point



agarose gel in 40 mM Tris-acetate, pH 8.5, 1 mM EDTA. The appropriate band can be excised from the gel, melted at 68°C and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Boulevard,  
5 Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a 1.05 kb SalI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SalI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli*  
10 XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment  
15 encoding a plant amino acid biosynthetic enzyme, and the 10 kD zein 3' region.

The chimeric gene described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0 to 1.5 mm long. The embryos are then placed  
20 with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al., (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can  
25 be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers  
30 resistance to herbicidal glutamine synthetase inhibitors such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from Cauliflower Mosaic Virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al., (1987) *Nature* 327:70-73) may be  
35 used to transfer genes to the callus culture cells. According to this method, gold particles (1 µm in diameter) are coated with DNA using the following technique. Ten µg of plasmid DNAs are added to 50 µL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 µL of a 2.5 M solution) and spermidine free base (20 µL of a 1.0 M solution) are added to the particles. The suspension is vortexed during the addition of these solutions.

After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200  $\mu$ L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30  $\mu$ L of ethanol. An aliquot (5  $\mu$ L) of the DNA-coated  
5 gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over agarose-  
10 solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture membrane that bursts when the He pressure in the shock  
15 tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains gluphosinate (2 mg per liter) and lacks casein or proline. The tissue continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing gluphosinate. After 6 weeks, areas of about 1 cm in diameter  
20 of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the  
25 tissue can be transferred to regeneration medium (Fromm et al., (1990) *Bio/Technology* 8:833-839).

### EXAMPLE 9

#### Expression of Chimeric Genes in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription  
30 terminator from the gene encoding the  $\beta$  subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant amino acid biosynthetic enzymes in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of  
35 phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone using appropriate oligonucleotide primers. Cloning sites can be

incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Plant amino acid biosynthetic enzymes are known to be localized in the chloroplasts.

5 Accordingly, for those enzymes (or polypeptides representing part of the instant amino acid biosynthetic enzymes) that lack a chloroplast targeting signal, the DNA fragment to be inserted into the expression vector can be synthesized by PCR with primers encoding a chloroplast targeting signal. For example, a chloroplast transit sequence equivalent to the cts of the small subunit of ribulose 1,5-bisphosphate carboxylase from soybean (Berry-Lowe et al. (1982) *J. Mol. Appl. Genet.* 1:483-498) may be used.

10 Soybean embryos may then be transformed with the expression vector comprising sequences encoding a plant amino acid biosynthetic enzyme. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar  
15 medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can maintained in 35 mL liquid media on a  
20 rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Kline et al. (1987) *Nature* (London) 327:70, U.S. Patent  
25 No. 4,945,050). A Du Pont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell et al.(1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid  
30 pJR225 (from *E. coli*; Gritz et al.(1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette comprising the phaseolin 5' region, the fragment encoding the biosynthetic enzyme and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the  
35 marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order): 5 µL DNA (1 µg/µL), 20 µl spermidine (0.1 M), and 50 µL CaCl<sub>2</sub> (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and

resuspended in 40  $\mu$ L of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five  $\mu$ L of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

#### EXAMPLE 10

##### Analysis of Amino Acid Content of the Seeds of Transformed Plants

To analyze for expression of the chimeric genes in seeds and for the consequences of expression on the amino acid content in the seeds, a seed meal can be prepared by any of a number of suitable methods known to those skilled in the art. The seed meal can be partially or completely defatted, via hexane extraction for example, if desired. Protein extracts can be prepared from the meal and analyzed for enzyme activity. Alternatively the presence of any of the expressed enzymes can be tested for immunologically by methods well-known to those skilled in the art. To measure free amino acid composition of the seeds, free amino acids can be extracted from the meal and analyzed by methods known to those skilled in the art (Bialeski et al. (1966) *Anal. Biochem.* 17:278-293). Amino acid composition can then be determined using any commercially available amino acid analyzer. To measure total amino acid composition of the seeds, meal containing both protein-bound and free amino acids can be acid hydrolyzed to release the protein-bound amino acids and the composition can then be determined using any commercially available amino acid analyzer. Seeds expressing the instant amino acid biosynthetic enzymes and with altered lysine, threonine, methionine, cysteine and/or isoleucine content as compared to the wild type seeds can thus be identified and propagated.

To measure free amino acid composition of the seeds, free amino acids can be extracted from 8-10 milligrams of the seed meal in 1.0 mL of methanol/chloroform/water

mixed in ratio of 12v/5v/3v (MCW) at room temperature. The mixture can be vortexed and then centrifuged in an eppendorf microcentrifuge for about 3 min; approximately 0.8 mL of supernatant is then decanted. To this supernatant, 0.2 mL of chloroform is added followed by 0.3 mL of water. The mixture is then vortexed and centrifuged in an eppendorf microcentrifuge for about 3 min. The upper aqueous phase, approximately 1.0 mL, can then be removed and dried down in a Savant Speed Vac Concentrator. The samples are then hydrolyzed in 6N hydrochloric acid, 0.4%  $\beta$ -mercaptoethanol under nitrogen for 24 h at 110-120°C. Ten percent of the sample can then be analyzed using a Beckman Model 6300 amino acid analyzer using post-column ninhydrin detection.

Relative free amino acid levels in the seeds are then compared as ratios of lysine, threonine, methionine, cysteine and/or isoleucine to leucine, thus using leucine as an internal standard.

### EXAMPLE 11

#### Expression of Chimeric Genes in Microbial Cells

The cDNAs encoding the instant plant amino acid biosynthetic enzymes can be inserted into the T7 *E. coli* expression vector pET24d (Novagen). Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the enzyme. This fragment may then be purified on a 1% NuSieve GTG™ low melting agarose gel (FMC). Buffer and agarose contain 10  $\mu$ g/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20  $\mu$ L of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs, Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pET24d is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pET24d and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar plates containing 2xYT media and 50  $\mu$ g/mL kanamycin. Transformants containing gene encoding the enzyme are then screened for the correct orientation with respect to pET24d T7 promoter by restriction enzyme analysis.

Clones in the correct orientation with respect to the T7 promoter can be transformed into BL21(DE3) competent cells (Novagen) and selected on 2xYT agar plates containing 50  $\mu$ g/ml kanamycin. A colony arising from this transformation construct can be grown overnight at 30°C in 2xYT media with 50  $\mu$ g/mL kanamycin. The culture is then diluted two fold with fresh media, allowed to re-grow for 1 h, and induced by adding isopropyl-thiogalactopyranoside to 1 mM final concentration. Cells are then harvested by centrifugation after 3 h and re-suspended in 50  $\mu$ L of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass

beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One  $\mu\text{g}$  of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

#### EXAMPLE 12

##### Evaluating Compounds for Their Ability to Inhibit the Activity of a Plant Amino Acid Biosynthetic Enzyme

The plant amino acid biosynthetic enzymes described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 6, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant enzymes may be expressed separately as mature proteins, or may be co-expressed in *E. coli* or another suitable expression background. In addition, whether expressed separately or in combination, the instant enzymes may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("His)<sub>6</sub>"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzymes. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the biosynthetic enzyme.

Purification of the instant enzymes, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the enzymes are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, an enzyme may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a (His)<sub>6</sub> peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include  $\beta$ -mercaptoethanol or other

reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the biosynthetic enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond™ affinity resin or other resin.

- 5        Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the plant amino acid biosynthetic enzymes disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. Examples of assays for many of these enzymes can be found in *Methods in*
- 10 *Enzymology* Vol. V, (Colowick and Kaplan eds.) Academic Press, New York or *Methods in Enzymology* Vol. XVII, (Tabor and Tabor eds.) Academic Press, New York. Specific examples may be found in the following references, each of which is incorporated herein by reference: dihydrodipicolinate reductase may be assayed as described in Farkas et al. (1965) *J. Biol. Chem.* 240: 4717-4722, or Cremer et al. (1988) *J. Gen. Microbiol.* 134:3221-3229;
- 15 diaminopimelate epimerase may be assayed as described in Work (1962) in *Methods in Enzymology* Vol. V, (Colowick and Kaplan eds.) 858-864, Academic Press, New York; threonine synthase may be assayed as described in Giovanelli et al. (1984) *Plant Physiol* 76: 285-292 or Curien et al. (1996) *FEBS Lett.* 390: 85-90; threonine deaminase may be assayed as described in Tomova et al. (1968) *Biochemistry (USSR)* 33: 200-208 or Dougall (1970)
- 20 *Phytochemistry* 9: 959-964; and S-adenosylmethionine synthetase may be assayed as described in Mudd (1960) *Biochim. Biophys. Acta* 38:354-355 or Boerjan et al. (1994) *Plant Cell* 6:1401-1414.

SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT:
  - (A) ADDRESSEE: E. I. DU PONT DE NEMOURS AND COMPANY
  - (B) STREET: 1007 MARKET STREET
  - (C) CITY: WILMINGTON
  - (D) STATE: DELAWARE
  - (E) COUNTRY: USA
  - (F) ZIP: 19898
  - (G) TELEPHONE: 302-992-4926
  - (H) TELEFAX: 302-773-0164
  - (I) TELEX: 6717325
- (ii) TITLE OF INVENTION: PLANT AMINO ACID BIOSYNTHETIC ENZYMES
- (iii) NUMBER OF SEQUENCES: 43
- (iv) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: DISKETTE, 3.50 INCH
  - (B) COMPUTER: IBM PC COMPATIBLE
  - (C) OPERATING SYSTEM: MICROSOFT WINDOWS 95
  - (D) SOFTWARE: MICROSOFT WORD VERSION 7.0A
- (v) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vi) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: 60/048,771
  - (B) FILING DATE: JUNE 6, 1997
- (vii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: MAJARIAN, WILLIAM R.
  - (B) REGISTRATION NUMBER: 41,173
  - (C) REFERENCE/DOCKET NUMBER: BB-1087



## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 908 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: csiln.pk0042.a3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

ACGCGGGACA GATAAGTGGC ATGGACGAGC CGCTGGAGAT CCCTGTGCTG AACGACCTCA      60
CCATGGTTCT GGGCTCCATA GCGCAGTCGA GAGCAACCGG CGTGGTGGTC GACTTCAGCG      120
AGCCTTCAGC TGTTTACGAC AATGTCAAGC AGGCAGCGGC GTTTGGTCTG AGCAGCGTCG      180
TCTACGTTCC GAAAATCGAG CTAGAGACAG TGA CTGAACT GTCAGCGTTC TCGGAGAAGG      240
CAAGCGGCTG CTTGGTTGCG CCAACGCTGT CGATTGGGTC CGTGCTCCTT CAGCAAGCGG      300
CTATACAGGC CTCGTTCCAC TACAGCAACG TTGAGATTGT GGAATCGAGA CCAAACCCAT      360
CGGATCTTCC ATCGCAAGAT GCAATCCAGA TTGCAAACAA CATATCAGAC CTTGGTCAGA      420
TATACAACAG GGAAGATATG GATTCCAGCA GTCCAGCCAG AGGCCAGCTG CTCGGGGAAG      480
ACGGAGTGCG CGTGACAGC ATGGTTCTCC CTGGTCTCGT CTCCAGCACG TCGATCAACT      540
TCTCTGGCCC AGGAGAGATG TACACCTTAC GGCATGACGT TGCGAATGTT CAGTGCCTGA      600
TGCCAGGACT GATCCTGGCG ATACGGAAGG TGGTGCGGTT CAAGAACTTG ATTTATGGGC      660
TAGAGAAGTT CTTGTAGTGA ACAACAAACA ACCAATGCAA AACATCGACA GGCAACAGGC      720
AAGGCAGATA TCATCTGACG TCGCAACAAC CAAAACGACA GAGATTGGA AAATAAAGGC      780
TGCACAGAAG ACGTCTGGGG TTTTGTGTGC ACCAGGCTGC GCAGAGAACG TCTGTCATTT      840
TGTGTGCACC ACTACGGCAC TACCTGCTGA GCGCGATTTT TATAAAAAAG GCATGGGAGG      900
GAGATCAT                                         908
  
```

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 224 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: csiln.pk0042.a3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Ala Gly Gln Ile Ser Gly Met Asp Glu Pro Leu Glu Ile Pro Val Leu
1           5           10           15
  
```

Asn Asp Leu Thr Met Val Leu Gly Ser Ile Ala Gln Ser Arg Ala Thr  
 20 25 30  
 Gly Val Val Val Asp Phe Ser Glu Pro Ser Ala Val Tyr Asp Asn Val  
 35 40 45  
 Lys Gln Ala Ala Ala Phe Gly Leu Ser Ser Val Val Tyr Val Pro Lys  
 50 55 60  
 Ile Glu Leu Glu Thr Val Thr Glu Leu Ser Ala Phe Cys Glu Lys Ala  
 65 70 75 80  
 Ser Gly Cys Leu Val Ala Pro Thr Leu Ser Ile Gly Ser Val Leu Leu  
 85 90 95  
 Gln Gln Ala Ala Ile Gln Ala Ser Phe His Tyr Ser Asn Val Glu Ile  
 100 105 110  
 Val Glu Ser Arg Pro Asn Pro Ser Asp Leu Pro Ser Gln Asp Ala Ile  
 115 120 125  
 Gln Ile Ala Asn Asn Ile Ser Asp Leu Gly Gln Ile Tyr Asn Arg Glu  
 130 135 140  
 Asp Met Asp Ser Ser Ser Pro Ala Arg Gly Gln Leu Leu Gly Glu Asp  
 145 150 155 160  
 Gly Val Arg Val His Ser Met Val Leu Pro Gly Leu Val Ser Ser Thr  
 165 170 175  
 Ser Ile Asn Phe Ser Gly Pro Gly Glu Met Tyr Thr Leu Arg His Asp  
 180 185 190  
 Val Ala Asn Val Gln Cys Leu Met Pro Gly Leu Ile Leu Ala Ile Arg  
 195 200 205  
 Lys Val Val Arg Phe Lys Asn Leu Ile Tyr Gly Leu Glu Lys Phe Leu  
 210 215 220

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 339 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: rls2.pk0017.d3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGATTGGCA GGAGAAATGC AGCAAAGGTC CTCTGCTCAA CGCAGATGCC GCCATCTCAG	60
AGCACAATCA AGGTTGTTAT CATTGGGGCG ACAAAGAGA TTGGAAGAAC GGCAATAGCG	120
GCAGTAAGTA AAGCAAGGGG AATGGAGCTT GCAGGGGCCA TAGATTCTCA GTGTATAGGC	180
CTAGATGCAG GAGAGATAAG TGGCATGGGA AGAACCTGG AAATTCCGGT GCTCAATGAT	240
CTCACAATGG TTCTGGGCTC AATTGCACAA ACCAGAGCAA CTGGAGTGGT GGTGATTTT	300
AGTGAACCTT CAACTGTTTA TGATAATGTC AACAGGCA	339

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 113 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: rls2.pk0017.d3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Lys Ile Gly Arg Arg Asn Ala Ala Lys Val Leu Cys Ser Thr Gln Met  
 1 5 10 15  
 Pro Pro Ser Gln Ser Thr Ile Lys Val Val Ile Ile Gly Ala Thr Lys  
 20 25 30  
 Glu Ile Gly Arg Thr Ala Ile Ala Ala Val Ser Lys Ala Arg Gly Met  
 35 40 45  
 Glu Leu Ala Gly Ala Ile Asp Ser Gln Cys Ile Gly Leu Asp Ala Gly  
 50 55 60  
 Glu Ile Ser Gly Met Gly Arg Thr Leu Glu Ile Pro Val Leu Asn Asp  
 65 70 75 80  
 Leu Thr Met Val Leu Gly Ser Ile Ala Gln Thr Arg Ala Thr Gly Val  
 85 90 95  
 Val Val Asp Phe Ser Glu Pro Ser Thr Val Tyr Asp Asn Val Lys Gln  
 100 105 110

Ala

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 275 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Synechocystus sp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Ala Asn Gln Asp Leu Ile Pro Val Val Val Asn Gly Ala Ala Gly  
 1 5 10 15  
 Lys Met Gly Arg Glu Val Ile Lys Ala Val Ala Gln Ala Pro Asp Leu  
 20 25 30  
 Gln Leu Val Gly Ala Val Asp His Asn Pro Ser Leu Gln Gly Gln Asp  
 35 40 45  
 Ile Gly Glu Val Val Gly Ile Ala Pro Leu Glu Val Pro Val Leu Ala  
 50 55 60

Asp Leu Gln Ser Val Leu Val Leu Ala Thr Gln Glu Lys Ile Gln Gly  
 65 70 75 80  
 Val Met Val Asp Phe Thr His Pro Ser Gly Val Tyr Asp Asn Val Arg  
 85 90 95  
 Ser Ala Ile Ala Tyr Gly Val Arg Pro Val Val Gly Thr Thr Gly Leu  
 100 105 110  
 Ser Glu Gln Gln Ile Gln Asp Leu Gly Asp Phe Ala Glu Lys Ala Ser  
 115 120 125  
 Thr Gly Cys Leu Ile Ala Pro Asn Phe Ala Ile Gly Val Leu Leu Met  
 130 135 140  
 Gln Gln Ala Ala Val Gln Ala Cys Gln Tyr Phe Asp His Val Glu Ile  
 145 150 155 160  
 Ile Glu Leu His His Asn Gln Lys Ala Asp Ala Pro Ser Gly Thr Ala  
 165 170 175  
 Ile Lys Thr Ala Gln Met Leu Ala Glu Met Gly Lys Thr Phe Asn Pro  
 180 185 190  
 Pro Ala Val Glu Glu Lys Glu Thr Ile Ala Gly Ala Lys Gly Gly Leu  
 195 200 205  
 Gly Pro Gly Gln Ile Pro Ile His Ser Ile Arg Leu Pro Gly Leu Ile  
 210 215 220  
 Ala His Gln Glu Val Leu Phe Gly Ser Pro Gly Gln Leu Tyr Thr Ile  
 225 230 235 240  
 Arg His Asp Thr Thr Asp Arg Ala Cys Tyr Met Pro Gly Val Leu Leu  
 245 250 255  
 Gly Ile Arg Lys Val Val Glu Leu Lys Gly Leu Val Tyr Gly Leu Glu  
 260 265 270  
 Lys Leu Leu  
 275

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1012 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: chp2.pk0008.h4
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TATTGCCAGA GATGTGTGGT AATGGAGTCC GTTGCTTCGC TCGGTTTATA GCCGAGATTG	60
AAAATCTGCA GGGGACAAAT AGATTCACTA TTCATACTGG TGCTGGAAAG ATCGTTCCTG	120
AAATACAAAG TGATGGGCAG GTAAAGGTTG ATATGGGCGA GCCTATCCTT TCTGGACTAG	180
ACATCCCCAC AAAACTGCTA GCTACCAAGA ACAAAGCTGT TGTTCAAGCT GAATTGGCAG	240
TTGAGGGCTT AACATGGCAT GTCACATGTG TTAGCATGGG AAACCCTCAC TGTGTCACAT	300

```

TTGGTGCAAA TGAGTTAAAG GTATTGCAGG TCGACGATTT AAAACTTAGC GAAATTGGGC      360
CTAAATTTGA GCATCATGAA ATGTTTCCTG CTCGCACAAA CACAGAATTC GTACAGGTTT      420
TGTCTCGCTC ACACCTCAAA ATGCGGGTCT GGGAACTGG TGCTGGAGCA ACTCTTGCCT      480
GTGGTACTGG TGCTTGTGCA GTGGTTGTTG CAGCTGTTCT TGAGGGTCGA GCTGAGCGGA      540
AATGTGTAGT TGATTTGCCT GCGGGGCCAT TGGAAATTGA GTGGAGGGAG GATGACAATC      600
ATGTTTACAT GACTGGTCCT GCAGAGGTCG TCTTTTATGG ATCTGTTGTT CACTAGGTAC      660
TGGGGACCAA GATAGAAGGG TTGGCTGCCA CTCAGAGCTT GTGAGATTGG TTATAGTATC      720
CATGAAACAG AGTGTTCCTG TACCAGTACA CTTGTTTCTG TATTCTTAAT TATGATTGCT      780
TGATTTGGGT AGCMGTAGAG GCTTCCTTTT GAAGCATTCT AGTGTTTCMCC TTTTGTACTC      840
CTTTAGTTTG TCAGGTTTGA AACTACATG GGTAACATGT CYTCCCACC ATTTTCYGT      900
TCTTTTCTTT GTAAGTGAAC GCCAATGCAG TTTTAGTATT GTTTTCTATA GATTGTCTT      960
GATGCACTGG GCTTACTACT TATTTTCTGG TATGAATGCT GCCTATTTCC TG      1012

```

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 217 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: chp2.pk0008.h4

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Leu Pro Glu Met Cys Gly Asn Gly Val Arg Cys Phe Ala Arg Phe Ile
1           5           10           15
Ala Glu Ile Glu Asn Leu Gln Gly Thr Asn Arg Phe Thr Ile His Thr
20           25           30
Gly Ala Gly Lys Ile Val Pro Glu Ile Gln Ser Asp Gly Gln Val Lys
35           40           45
Val Asp Met Gly Glu Pro Ile Leu Ser Gly Leu Asp Ile Pro Thr Lys
50           55           60
Leu Leu Ala Thr Lys Asn Lys Ala Val Val Gln Ala Glu Leu Ala Val
65           70           75           80
Glu Gly Leu Thr Trp His Val Thr Cys Val Ser Met Gly Asn Pro His
85           90           95
Cys Val Thr Phe Gly Ala Asn Glu Leu Lys Val Leu Gln Val Asp Asp
100          105          110
Leu Lys Leu Ser Glu Ile Gly Pro Lys Phe Glu His His Glu Met Phe
115          120          125
Pro Ala Arg Thr Asn Thr Glu Phe Val Gln Val Leu Ser Arg Ser His
130          135          140

```

Leu Lys Met Arg Val Trp Glu Arg Gly Ala Gly Ala Thr Leu Ala Cys  
 145 150 155 160  
 Gly Thr Gly Ala Cys Ala Val Val Val Ala Ala Val Leu Glu Gly Arg  
 165 170 175  
 Ala Glu Arg Lys Cys Val Val Asp Leu Pro Gly Gly Pro Leu Glu Ile  
 180 185 190  
 Glu Trp Arg Glu Asp Asp Asn His Val Tyr Met Thr Gly Pro Ala Glu  
 195 200 205  
 Val Val Phe Tyr Gly Ser Val Val His  
 210 215

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 481 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: rls48.pk0036.h10
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGTATCCGGC GCCGACGGTG TGATCTTCGT CATGCCGGGG GTCAATGGCG CGGACTACAC 60  
 CATGAGGATC TTCAACTCGG ACGGCAGTGA GCCGGAGATG TGTGGCAATG GAGTCCGTTG 120  
 CTTTGCCCCG TTTATAGCTG AGCTTGAAAA CCTACAGGGA ACACATAGCT TCAAATTCA 180  
 CACTGGCGCT GGGCTAATCA TTCCTGAAAT ACAAATGAT GGCAAGGTAA AGGTTGATAT 240  
 GGGCCAGCCC ATTCTCTCTG GACCAGATAT TCCAACAAAA CTGCCATCCA CCAAGAATGA 300  
 AGCCGTTGTC CAAGCTGATT TGGGCAGTTG ATGGCTCAAC ATGGCAAGTA ACCTGTGTTA 360  
 GCATGGGCAA TCCACATTGT GTCACATTG GCACAAAGGA GCTCAAGGTT TTGCATGTTG 420  
 ATGATTAAAG CTTAATGATA TTGGGGCCTA AATTCAGCAT CATGAAATGT TCCTGCCCCA 480  
 C 481

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 85 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: not relevant
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: rls48.pk0036.h10
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Val Ser Gly Ala Asp Gly Val Ile Phe Val Met Pro Gly Val Asn Gly  
 1 5 10 15

Ala Asp Tyr Thr Met Arg Ile Phe Asn Ser Asp Gly Ser Glu Pro Glu  
 20 25 30  
 Met Cys Gly Asn Gly Val Arg Cys Phe Ala Arg Phe Ile Ala Glu Leu  
 35 40 45  
 Glu Asn Leu Gln Gly Thr His Ser Phe Lys Ile His Thr Gly Ala Gly  
 50 55 60  
 Leu Ile Ile Pro Glu Ile Gln Asn Asp Gly Lys Val Lys Val Asp Met  
 65 70 75 80  
 Gly Gln Pro Ile Leu  
 85

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1301 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATCCCTTATT AAGCAGGGGT TTCGCGGCGC GAGACGGTGA CACTGGCAGA GTGGAATTTT 60  
 CGCCGCCATT CGAAGCTACA GCGATGGCCA TAACCGCCAC CATTTCCGTT CCCCTCACAT 120  
 CCCCCAGTCG CCGCACTCTC ACCTCCGTCA ATAGCCTCTC TCCCCTTTCT ACCCGATCCA 180  
 CTTTGCCAC ACCGCAACGC ACTTTCAAAT ACCCTAATTC GCGCCTCGTC GTGTCTTCCA 240  
 TGAGACCCGA AACAGCCGTC AAAACTTCAT CCGCCTCCTT CCTCAACCGC AAGGAGTCCG 300  
 GCTTCCTCCA TTTCGCCAAG TACCACGGCC TCGGAAACGA CTCGTTTTG ATTGACAATA 360  
 GAGACTCCTC CGAGCCCAAG ATCAGTGCTG AGAAAGCGGT GCAACTGTGT GATCGGAACT 420  
 TCGGCGTTGG AGCTGACGGA GTTATCTTTG TCTTGCCTGG CATCAGTGGC ACCGATTATA 480  
 CCATGAGGAT TTTTAACTCT GATGGTAGTG AGCCTGAGAT GTGTGGCAAT GGAGTTCGAT 540  
 GCTTTGCCAA ATTTGTTTCT CAGCTTGAGA ATTTACATGG GAGGCATAGT TTTACCATTC 600  
 ATACTGGTGC TGGTCTGATT ATTCCTGAAG TCTTGAGGA TGGAAATGTC AGAGTTGATA 660  
 TGGGGGAGCC AGTTCTTAAA GCCTTGATG TGCCTACTAA ATTACCTGCA AATAAGGATA 720  
 ATGCTGTTGT TAAATCACAG CTAGTTGTAG ATGGAGTTAT TTGGCATGTG ACCTGTGTTA 780  
 GCATGGGGAA TCCACACTGT GTAACCTTCA GTAGAGAAGG AAGCCAGAAT TTGCTTGTTG 840  
 ATGAATTGAA GCTAGCAGAA ATTGGGCCAA AATTTGAACA TCATGAGGTG TTCCCTGCAC 900  
 GAACTAACAC AGAGTTTGTG CAAGTATTAT CTAACCTCTCA CTTGAAAATG CGTGTGTTGG 960  
 AGCGGGGAGC AGGAGCAACC CTAGCCTGTG GAACTGGAGC TTGTGCTACT GTTGTTGCAG 1020  
 CAGTTCTTGA GGGTCGTGCT GGGAGGAATT GCACGGTTGA TCTACCTGGA GGGCCTCTTC 1080  
 AGATTGAGTG GAGGGAGGAA GATAATCATG TTTATATGAC AGGCTCAGCC GATGTAGTTT 1140

ATTATGGTTC TTTGCCCCCTT TGATATGTTG CCCCCATTGT TAAACCCAAT ATGGAATTAG 1200  
 GAATTGGTGA ATAATATTTG TATGAGAGGT GGACTTTCTG CTTGTTCTTA ATATTTTGCC 1260  
 ACGTCTTTAT AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A 1301

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 359 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Ala Ile Thr Ala Thr Ile Ser Val Pro Leu Thr Ser Pro Ser Arg  
 1 5 10 15  
 Arg Thr Leu Thr Ser Val Asn Ser Leu Ser Pro Leu Ser Thr Arg Ser  
 20 25 30  
 Thr Leu Pro Thr Pro Gln Arg Thr Phe Lys Tyr Pro Asn Ser Arg Leu  
 35 40 45  
 Val Val Ser Ser Met Ser Thr Glu Thr Ala Val Lys Thr Ser Ser Ala  
 50 55 60  
 Ser Phe Leu Asn Arg Lys Glu Ser Gly Phe Leu His Phe Ala Lys Tyr  
 65 70 75 80  
 His Gly Leu Gly Asn Asp Phe Val Leu Ile Asp Asn Arg Asp Ser Ser  
 85 90 95  
 Glu Pro Lys Ile Ser Ala Glu Lys Ala Val Gln Leu Cys Asp Arg Asn  
 100 105 110  
 Phe Gly Val Gly Ala Asp Gly Val Ile Phe Val Leu Pro Gly Ile Ser  
 115 120 125  
 Gly Thr Asp Tyr Thr Met Arg Ile Phe Asn Ser Asp Gly Ser Glu Pro  
 130 135 140  
 Glu Met Cys Gly Asn Gly Val Arg Cys Phe Ala Lys Phe Val Ser Gln  
 145 150 155 160  
 Leu Glu Asn Leu His Gly Arg His Ser Phe Thr Ile His Thr Gly Ala  
 165 170 175  
 Gly Leu Ile Ile Pro Glu Val Leu Glu Asp Gly Asn Val Arg Val Asp  
 180 185 190  
 Met Gly Glu Pro Val Leu Lys Ala Leu Asp Val Pro Thr Lys Leu Pro  
 195 200 205  
 Ala Asn Lys Asp Asn Ala Val Val Lys Ser Gln Leu Val Val Asp Gly  
 210 215 220  
 Val Ile Trp His Val Thr Cys Val Ser Met Gly Asn Pro His Cys Val  
 225 230 235 240  
 Thr Phe Ser Arg Glu Gly Ser Gln Asn Leu Leu Val Asp Glu Leu Lys  
 245 250 255



Leu Ala Glu Ile Gly Pro Lys Phe Glu His His Glu Val Phe Pro Ala  
 260 265 270  
 Arg Thr Asn Thr Glu Phe Val Gln Val Leu Ser Asn Ser His Leu Lys  
 275 280 285  
 Met Arg Val Trp Glu Arg Gly Ala Gly Ala Thr Leu Ala Cys Gly Thr  
 290 295 300  
 Gly Ala Cys Ala Thr Val Val Ala Ala Val Leu Glu Gly Arg Ala Gly  
 305 310 315 320  
 Arg Asn Cys Thr Val Asp Leu Pro Gly Gly Pro Leu Gln Ile Glu Trp  
 325 330 335  
 Arg Glu Glu Asp Asn His Val Tyr Met Thr Gly Ser Ala Asp Val Val  
 340 345 350  
 Tyr Tyr Gly Ser Leu Pro Leu  
 355

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 602 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:
- (B) CLONE: wlm24.pk0030.g4

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTCCACCGCC CCCTCCTCGG GCGGTCGCCT CCTCCGTCCG TTCTGTGGGA ATCCGCGCCC 60  
 CCGCCGCGCC GTCGCCTCGA TGGCCGTGTC CGCTCCCAAG TCGCCAGCCG CCGCCTCGTT 120  
 CCTCGAGCGC CGCGAGTCCG AGCGCGCGCT CCACTTCGTG AAGTACCAGG GCCTCGGCAA 180  
 CGACTTCATA ATGGTCGACA ACAGGGATTC GGCCGTACCG AAGGTGACAC CGGAGGAGGC 240  
 GGCGAAGCTA TGCGACCGAA ACTTTGGGTA TTGGGTGCTG ATGGCGTCAT CTTGCTCCTG 300  
 CCGGGGGTCA ACGGCGCGGA CTACACTATG AGGATATTCA ACTCCGATGG CAGCAACCGG 360  
 AATGTNTGGN ATGGATTCTG TGCTTGCTCG CTTTATACGG AGTTGAAATC TACANGGAAA 420  
 CATACTTCAA AACAANAGGG GGCTGGATTA ATATCCTGAA ATANANACAT GNAAGTTANG 480  
 TNATATGGGC AACAATCTTA TGGCANATTT CANAAAATGC ATCACAAGAT AACTTNTAAA 540  
 ACGATTGAAT TAGGCAANAG AANTACCGTT ATAGGAACCC ATGAANCTTG TNAAATTAAG 600  
 GT 602

## (2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 80 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: not relevant
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:  
(B) CLONE: wlm24.pk0030.g4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```

Ala Leu His Phe Val Lys Tyr Gln Gly Leu Gly Asn Asp Phe Ile Met
1           5           10           15
Val Asp Asn Arg Asp Ser Ala Val Pro Lys Val Thr Pro Glu Glu Ala
20           25           30
Ala Lys Leu Cys Asp Arg Asn Phe Gly Xaa Gly Ala Asp Gly Val Ile
35           40           45
Phe Val Leu Pro Gly Val Asn Gly Ala Asp Tyr Thr Met Arg Ile Phe
50           55           60
Asn Ser Asp Gly Ser Asn Arg Asn Val Trp Xaa Gly Phe Val Ala Cys
65           70           75           80

```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 279 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: not relevant  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Synechocystus sp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

Met Ala Leu Ser Phe Ser Lys Tyr His Gly Leu Gly Asn Asp Phe Ile
1           5           10           15
Leu Val Asp Asn Arg Gln Ser Thr Glu Pro Cys Leu Thr Pro Asp Gln
20           25           30
Ala Gln Gln Leu Cys Asp Arg His Phe Gly Ile Gly Ala Asp Gly Val
35           40           45
Ile Phe Ala Leu Pro Gly Gln Gly Gly Thr Asp Tyr Thr Met Arg Ile
50           55           60
Phe Asn Ser Asp Gly Ser Glu Pro Glu Met Cys Gly Asn Gly Ile Arg
65           70           75           80
Cys Leu Ala Lys Phe Leu Ala Asp Leu Glu Gly Val Glu Glu Lys Thr
85           90           95
Tyr Arg Ile His Thr Leu Ala Gly Val Ile Thr Pro Gln Leu Leu Ala
100          105          110
Asp Gly Gln Val Lys Val Asp Met Gly Glu Pro Gln Leu Leu Ala Glu
115          120          125
Leu Ile Pro Thr Thr Leu Ala Pro Ala Gly Glu Lys Val Val Asp Leu
130          135          140
Pro Leu Ala Val Ala Gly Gln Thr Trp Ala Val Thr Cys Val Ser Met
145          150          155          160

```

Gly Asn Pro His Cys Leu Thr Phe Val Asp Asp Val Asp Ser Leu Asn  
 165 170 175  
 Leu Thr Glu Ile Gly Pro Leu Phe Glu His His Pro Gln Phe Ser Gln  
 180 185 190  
 Arg Thr Asn Thr Glu Phe Ile Gln Val Leu Gly Ser Asp Arg Leu Lys  
 195 200 205  
 Met Arg Val Trp Glu Arg Gly Ala Gly Ile Thr Leu Ala Cys Gly Thr  
 210 215 220  
 Gly Ala Cys Ala Thr Val Val Ala Ala Val Leu Thr Gly Arg Gly Asp  
 225 230 235 240  
 Arg Arg Cys Thr Val Glu Leu Pro Gly Gly Asn Leu Glu Ile Glu Trp  
 245 250 255  
 Ser Ala Gln Asp Asn Arg Leu Tyr Met Thr Gly Pro Ala Gln Arg Val  
 260 265 270  
 Phe Ser Gly Gln Ala Glu Ile  
 275

## (2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1160 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: cc2.pk0031.c9

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTCGGCTGCG CGTCCACGGG AGACACCTCC GCCGCGCTCT CGGCCTACTG CGCAGCCGCG	60
GGAATCCCCG CCATCGTGTT CCTGCCAGCG GACCGCATCT CGCTGCAGCA GCTCATCCAG	120
CCGATCGCCA ACGGCGCCAC CGTGCTCTCT CTAGACACTG ATTTTGATGG CTGCATGCGG	180
CTCATTCGCG AGGTCACTGC AGAGCTGCCA ATCTACCTTG CCAATTCGCT CAACCCGCTC	240
CGCCTTGAGG GGCAGAAGAC AGCGGCCATC GAGATATTGC AGCAGTTCAA TTGGCAGGTG	300
CCAGATTGGG TCATTGTTCC AGGAGGCAAT CTTGGGAATA TCTATGCATT CTACAAGGGG	360
TTTGAGATGT GCCGCGTTCT TGGACTTGTT GATCGCGTGC CACGGCTTGT CTGCGCACAG	420
GCTGCAAATG CAAATCCATT GTACCGGTAC TACAAGTCAG GTTGACTGA GTTTGAGCCA	480
CAAATGCCC AGACTACATT TGCATCTGCG ATACAGATTG GTGATCCTGT ATCTGTTGAC	540
CGTGCGGTGG TCGCGCTGAA GGCCACTGAC GGTATTGTGG AGGAGGCTAC AGAGGAGGAG	600
CTAATGGATG CAACGGCGCT TGCTGACCGC ACTGGGATGT TTGCTTGCCC ACATACTGGG	660
GTTGCACTTG CTGCTTTGTT TAAGCTTCAG GGTGAGCGTA TAATTGGCCC TAATGACCGC	720
ACTGTGGTTG TTAGCACAGC TCATGGGCTG AAGTTCACGC AGTCAAAGAT TGACTIONCAT	780

GACAAAAACA TCAAAGACAT GGTTCGCCAG TATGCTAATC CACCGATCAG TGTGAAGGCT 840  
 GACTTTGGTT CTGTGATGGA TGTCTCCAG AAAAATCTCA ATGGTAAGAT ATAAAGTTAT 900  
 ATGATTAATT AACCCCTCAA ACTGTTTTTT TTTGTTTTTT CGTTCAGGA ATTTTATTCC 960  
 TGAGTCTTTC AACTTTGTTT GGTGAACATG GTATGGTGCT AAAATCTAGA CCTAATACCT 1020  
 TGTAGTACTA GTTCTGGAGG CTCTTTTGGT TGTAGGTCGA AGTGGATAGA GCTGTTTCCTT 1080  
 GTACTTTATC TGTTTCATGT AATATGAATA ATAAATTATG GTCTAAATAT TTGAATAAAA 1140  
 AATCGTTTGG AATGACCCAC 1160

## (2) INFORMATION FOR SEQ ID NO:16:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 297 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: cc2.pk0031.c9

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Val Gly Cys Ala Ser Thr Gly Asp Thr Ser Ala Ala Leu Ser Ala Tyr  
 1 5 10 15  
 Cys Ala Ala Ala Gly Ile Pro Ala Ile Val Phe Leu Pro Ala Asp Arg  
 20 25 30  
 Ile Ser Leu Gln Gln Leu Ile Gln Pro Ile Ala Asn Gly Ala Thr Val  
 35 40 45  
 Leu Ser Leu Asp Thr Asp Phe Asp Gly Cys Met Arg Leu Ile Arg Glu  
 50 55 60  
 Val Thr Ala Glu Leu Pro Ile Tyr Leu Ala Asn Ser Leu Asn Pro Leu  
 65 70 75 80  
 Arg Leu Glu Gly Gln Lys Thr Ala Ala Ile Glu Ile Leu Gln Gln Phe  
 85 90 95  
 Asn Trp Gln Val Pro Asp Trp Val Ile Val Pro Gly Gly Asn Leu Gly  
 100 105 110  
 Asn Ile Tyr Ala Phe Tyr Lys Gly Phe Glu Met Cys Arg Val Leu Gly  
 115 120 125  
 Leu Val Asp Arg Val Pro Arg Leu Val Cys Ala Gln Ala Ala Asn Ala  
 130 135 140  
 Asn Pro Leu Tyr Arg Tyr Tyr Lys Ser Gly Trp Thr Glu Phe Glu Pro  
 145 150 155 160  
 Gln Thr Ala Glu Thr Thr Phe Ala Ser Ala Ile Gln Ile Gly Asp Pro  
 165 170 175  
 Val Ser Val Asp Arg Ala Val Val Ala Leu Lys Ala Thr Asp Gly Ile  
 180 185 190

Val Glu Glu Ala Thr Glu Glu Glu Leu Met Asp Ala Thr Ala Leu Ala  
 195 200 205

Asp Arg Thr Gly Met Phe Ala Cys Pro His Thr Gly Val Ala Leu Ala  
 210 215 220

Ala Leu Phe Lys Leu Gln Gly Gln Arg Ile Ile Gly Pro Asn Asp Arg  
 225 230 235 240

Thr Val Val Val Ser Thr Ala His Gly Leu Lys Phe Thr Gln Ser Lys  
 245 250 255

Ile Asp Tyr His Asp Lys Asn Ile Lys Asp Met Val Cys Gln Tyr Ala  
 260 265 270

Asn Pro Pro Ile Ser Val Lys Ala Asp Phe Gly Ser Val Met Asp Val  
 275 280 285

Leu Gln Lys Asn Leu Asn Gly Lys Ile  
 290 295

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 325 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: csl.pk0058.g5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGGCTTGCA AGTACTCCAA CCCGCCTGTG AGCGTGAAGG CTGACTTTGG CGCCGTGATG 60  
 GATGTGCTGA AGAAGAGGCT CAAGGGCAAG CTCTGAGCGC CTGTGCCTGG CTAATGCAAT 120  
 CAACTGATTG GAATGCAGTG GTTTCGTCGG TATCGGGGGG TCTTTTAGGC TTCAGAAATT 180  
 CTGTCTGGGT TAGACTATTT GTTTGTGGAG TTTAGCAGGA GAATGGCTAT CTCTCCTGCA 240  
 AGACTGGCGC TCTTTCTTGT GCTACGAATG TGTTACCATG GATAATAAGT GTAGTCGCTG 300  
 TCGGATTGAA TAATCAAAAA AAAAN 325

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 31 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: csl,pk0058.g5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Met Ala Cys Lys Tyr Ser Asn Pro Pro Val Ser Val Lys Ala Asp Phe  
 1 5 10 15

Gly Ala Val Met Asp Val Leu Lys Lys Arg Leu Lys Gly Lys Leu  
 20 25 30

## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 528 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: rls72.pk0018.e7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

ACACCCAACA CGCAGACTTG ACAGATTCTG CTACTACAAA TCCTGCATAT TTAACAGCGC      60
TGCAACTCGA CGATGGAGAA CGGTGCTGCA ACCAACGGGG CGTCGGAGAA GTCGCACTCT      120
CCTTCACAGA CCTACCTCTC CACAAGGGGA GACGATTATG GGCTCTCATT CGAGACCGTC      180
GTCCTCAAAG GTCTTGCGGC TGACGGGGGT CTTTTCCTGC CCGAGGAAGT GCGCGCGGCA      240
ACCGAGTGGC AAAGCTGGAA AGACCTGCCC TACACCGAGC TTGCCGTCAA GGTTCCTCAGC      300
TTGTACATCT CCCCCGCCGA GGTGCCGACG GAAGACCTCA GGGCGCTCGT CGAGCGCAGC      360
TACTCGACCT TCCGATCCAA GGAGGTTGTG CCGCTGGTGA AGCTGGAGGA CAACCTTCAC      420
CTGCTGGAGC TATTCCACGG CCCCAACTAC TCGTTCAAGG ACTGCGCGCT GCAATTCCTT      480
GGTAACCTCN TCGAGTACTT TTGACTCNCA AGAACAAGGG AAAGGAGG      528
  
```

## (2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 143 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: rls72.pk0018.e7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

Met Glu Asn Gly Ala Ala Thr Asn Gly Ala Ser Glu Lys Ser His Ser
1      5      10      15
Pro Ser Gln Thr Tyr Leu Ser Thr Arg Gly Asp Asp Tyr Gly Leu Ser
20     25     30
Phe Glu Thr Val Val Leu Lys Gly Leu Ala Ala Asp Gly Gly Leu Phe
35     40     45
Leu Pro Glu Glu Val Pro Ala Ala Thr Glu Trp Gln Ser Trp Lys Asp
50     55     60
Leu Pro Tyr Thr Glu Leu Ala Val Lys Val Leu Ser Leu Tyr Ile Ser
65     70     75     80
  
```

Pro Ala Glu Val Pro Thr Glu Asp Leu Arg Ala Leu Val Glu Arg Ser  
                             85                            90                            95

Tyr Ser Thr Phe Arg Ser Lys Glu Val Val Pro Leu Val Lys Leu Glu  
                             100                            105                            110

Asp Asn Leu His Leu Leu Glu Leu Phe His Gly Pro Asn Tyr Ser Phe  
                             115                            120                            125

Lys Asp Cys Ala Leu Gln Phe Leu Gly Asn Leu Xaa Glu Tyr Phe  
                             130                            135                            140

## (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 571 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: sel.06a03

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

GGATGCAATG GTGCAGGCTG ATTCCACTGG AATGTTTATA TGTCCACACA CTGGGGTGGC      60
TCTGGCGGCG CTTATTAAGC TGAGGAATCG TGGGGTTATC GGTGCCGGTG AGAGGGTTGT      120
GGTGGTGAGC ACTGCACATG GATTGAAGTT TGCACAGAGC AAGATTGATT ATCATTCTGG      180
GCTCATTCCT GGAATGGGCC GCTATGCTAA CCCGCTGGTT TCGGTTAAGG CGGATTTTGG      240
ATCGGTCATG GATGTTCTCA AGGATTCTTG CACAACAAGT CCCCCGACTT TAACAAGTCT      300
TGACGTTGCC AAGTAAGTTT TAGTTCGGGG TTTTCTCTGA TTAAAGATGT TTTTAAACAT      360
GTTTGTGTNC ACTTTCGGTC GTTATTATGG ATTTGTAAGA TTGGGCCCAA GTATTTCGAGG      420
GTTTGATTTC AAACAACATG CTTCTGGTGA CGCAATGCAA ATTTCCGNGC ATAACATCAT      480
TGTCGAAGAT GGATCNCGAC CGATGAAACT GTGTGGCAAG TAATGAGAAG AAAATAGGGC      540
ACTTGACAG AGATTTNAAA GNTTAATTTC N      571

```

## (2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 104 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: sel.06a03

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Asp Ala Met Val Gln Ala Asp Ser Thr Gly Met Phe Ile Cys Pro His  
 1                            5                            10                            15

Thr Gly Val Ala Leu Ala Ala Leu Ile Lys Leu Arg Asn Arg Gly Val  
 20 25 30  
 Ile Gly Ala Gly Glu Arg Val Val Val Val Ser Thr Ala His Gly Leu  
 35 40 45  
 Lys Phe Ala Gln Ser Lys Ile Asp Tyr His Ser Gly Leu Ile Pro Gly  
 50 55 60  
 Met Gly Arg Tyr Ala Asn Pro Leu Val Ser Val Lys Ala Asp Phe Gly  
 65 70 75 80  
 Ser Val Met Asp Val Leu Lys Asp Ser Cys Thr Thr Ser Pro Pro Thr  
 85 90 95  
 Leu Thr Ser Leu Asp Val Ala Lys  
 100

## (2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2191 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: srl.pk0003.f6

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GCTTCCTCTT CTCTGTTTCA GTCTCTCCCT TTCTCTCTCC AACCTCTAA ACCCTACGCG 60  
 CCTCCCAAAC CCGCCGCCCA CTTGCTTGTC CGCGCCCAAT CCCCCCTCAC TCAGAACAAC 120  
 AACTCCTCCT CCAAGCATCG CCGCCCCGCC GACGAGAACA TCCGCGACGA GGCCCGCCGC 180  
 ATCAATGCGC CCCACGACCA CCACCTCTTC TCGGCCAAGT ACGTCCCCTT CAACGCCGAC 240  
 TCCTCCTCCT CCTCCTCCAC GGAGTCCTAC TCGCTCGACG AGATCGTCTA CCGCTCCCAA 300  
 TCCGGCGGCC TCCTGGACGT CCAGCACGAC ATGGATGCCC TCAAGCGTTT CGACGGCGAG 360  
 TACTGGCGCA ACCTCTTCGA CTCGCGCGTG GGCAAAACCA CCTGGCCTTA CGGCTCCGGC 420  
 GTCTGGAGCA AAAAAGAATG GGTCCTCCCC GAGATCCACG ACGACGATAT CGTCTCCGCC 480  
 TTCGAGGGTA ACTCCAACCT CTTCTGGGCC GAGCGTTTCG GCAAACAGTT CCTCGGCATG 540  
 AACGATTTGT GGGTCAAACA CTGCGGAATC AGCCACACGG GCAGCTTCAA GGATCTCGGC 600  
 ATGACCGTCC TCGTCAGCCA GGTCAATCGC TTGAGAAAAA TGAACCGCCC CGTCGTCGGT 660  
 GTTGGTTGCG CCTCCACCGG TGACACATCG GCCGCTTTAT CCGCCTATTG CGCTTCCGCT 720  
 GCCATTCTTT CCATTGTGTT TTTGCCTGCT AATAAAATCT CTCTTGCCCA ACTTGTTTCA 780  
 CCTATTGCCA ATGGAGCCTT TGTGTTGAGT ATCGACACTG ATTTTGATGG TTGCATGCAG 840  
 TTGATCAGAG AAGTCACTGC TGAATTGCCT ATTTATTTGG CTAACCTCTT CAACAGTTTG 900  
 AAGTTGGAAG GGCAGAAAAC TGCTGCTATT GAGATTCTGC AGCAGTTTGA TTGGCAGGTT 960  
 CCTGATTGGG TCATTGTGCC TGGAAGCAAC CTTGGCAACA TTTATGCCTT TTACAAAGGG 1020



```

TTTAAGATGT TTCAAGAGCT TGGGCTTGTG GATAAGATTC CAAGGCTTGT TTGTGCTCAG 1080
GCTGCCAATG CTGATCCTTT GTATTTGTAC TTTAAATCCG GGTGGAAGGA GTTTAAGCCT 1140
GTGAAGTCGA GCACTACATT TGCTTCTGCC ATTCAAATTG GTGATCCTGT TTCCATTGAC 1200
AGGGCGGTTC ACGCGCTAAA GAGTTGCGAT GGGATTGTGG AGGAGGCCAC GGAGGAGGAG 1260
TTGATGGATG CTACAGCGCA GCGGATTCT ACTGGGATGT TTATTTGCCC CCACACCGGG 1320
GTTGCTTTAA CTGCATTGTT TAAGCTCAGG AACAGCGGGG TTATTAAGGC CACTGATAGG 1380
ACTGTGGTGG TTAGCACTGC TCATGGCTTG AAGTTCAC TC AGTCCAAGAT TGATTACCAT 1440
TCTAAGGACA TCAAGGACAT GGCTTGCCGC TATGCTAACC CGCCCATGCA AGTGAAGGCA 1500
GACTTTGGCT CGGTTATGGA TGTTTTGAAG ACGTATTTGC AGAGTAAGGC TCATTAGGTT 1560
AGCATTGCAA GTTTTGCTCC TCCTGAGTTT GCTCATTATT TACTTACTTT TAGGCACTAC 1620
TGCTGTATTG TCTTTTCTAT GAGCTAGGTT TGAGTGTTGT AATAATTTGC TTGCTGCATT 1680
ATGTATGCCG TCTAGTGTT CATATTGGGC ATCATCCTTA GTATTTGTTG TAGATTTTCT 1740
TTGCTGAGCA TTTGATATAA TAGCTCAAGT AGGAAAATGA ATTGGGTACT ATGAGGAATG 1800
CATATCATTG GCTTGTTATT ACTGGATTCC AGACCACCCC AAAAGAAAAT AATTCCAAAA 1860
AATATAATTA GAACAAATTT CGTCCTTGTT ATGCTGTTGG CATTAAGCTC AGTGTGGGTA 1920
TTACCAAGCA ACTCGAAATC AAGAGAAAAA AAAATTGACA GCAAAGGAGC TGCATTGTTG 1980
GACTGAGTCA CATCACTTCA TTGCTATGTC GTCATATTTT GTTGAATTAC GGGAAGGCAG 2040
CATGCACAGC AATATGCAGC GATTAAGTGA AGCCACACCG CACACATTGA AGTAGTAGTC 2100
AATTTAGACA CTCCATCTTG TACTTTCTAC AAAAATGAAT TTTTCTTAGC CATTAAGTAT 2160
AATATTTTAT TCTAAAAAAA AAAAAAAAAA A 2191

```

## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 518 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: srl.pk0003.f6

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```

Ala Ser Ser Ser Leu Phe Gln Ser Leu Pro Phe Ser Leu Gln Thr Ser
1           5           10           15
Lys Pro Tyr Ala Pro Pro Lys Pro Ala Ala His Phe Val Val Arg Ala
20           25           30
Gln Ser Pro Leu Thr Gln Asn Asn Asn Ser Ser Ser Lys His Arg Arg
35           40           45

```

Pro Ala Asp Glu Asn Ile Arg Asp Glu Ala Arg Arg Ile Asn Ala Pro  
 50 55 60  
 His Asp His His Leu Phe Ser Ala Lys Tyr Val Pro Phe Asn Ala Asp  
 65 70 75 80  
 Ser Ser Ser Ser Ser Ser Thr Glu Ser Tyr Ser Leu Asp Glu Ile Val  
 85 90 95  
 Tyr Arg Ser Gln Ser Gly Gly Leu Leu Asp Val Gln His Asp Met Asp  
 100 105 110  
 Ala Leu Lys Arg Phe Asp Gly Glu Tyr Trp Arg Asn Leu Phe Asp Ser  
 115 120 125  
 Arg Val Gly Lys Thr Thr Trp Pro Tyr Gly Ser Gly Val Trp Ser Lys  
 130 135 140  
 Lys Glu Trp Val Leu Pro Glu Ile His Asp Asp Asp Ile Val Ser Ala  
 145 150 155 160  
 Phe Glu Gly Asn Ser Asn Leu Phe Trp Ala Glu Arg Phe Gly Lys Gln  
 165 170 175  
 Phe Leu Gly Met Asn Asp Leu Trp Val Lys His Cys Gly Ile Ser His  
 180 185 190  
 Thr Gly Ser Phe Lys Asp Leu Gly Met Thr Val Leu Val Ser Gln Val  
 195 200 205  
 Asn Arg Leu Arg Lys Met Asn Arg Pro Val Val Gly Val Gly Cys Ala  
 210 215 220  
 Ser Thr Gly Asp Thr Ser Ala Ala Leu Ser Ala Tyr Cys Ala Ser Ala  
 225 230 235 240  
 Ala Ile Pro Ser Ile Val Phe Leu Pro Ala Asn Lys Ile Ser Leu Ala  
 245 250 255  
 Gln Leu Val Gln Pro Ile Ala Asn Gly Ala Phe Val Leu Ser Ile Asp  
 260 265 270  
 Thr Asp Phe Asp Gly Cys Met Gln Leu Ile Arg Glu Val Thr Ala Glu  
 275 280 285  
 Leu Pro Ile Tyr Leu Ala Asn Ser Leu Asn Ser Leu Lys Leu Glu Gly  
 290 295 300  
 Gln Lys Thr Ala Ala Ile Glu Ile Leu Gln Gln Phe Asp Trp Gln Val  
 305 310 315 320  
 Pro Asp Trp Val Ile Val Pro Gly Ser Asn Leu Gly Asn Ile Tyr Ala  
 325 330 335  
 Phe Tyr Lys Gly Phe Lys Met Phe Gln Glu Leu Gly Leu Val Asp Lys  
 340 345 350  
 Ile Pro Arg Leu Val Cys Ala Gln Ala Ala Asn Ala Asp Pro Leu Tyr  
 355 360 365  
 Leu Tyr Phe Lys Ser Gly Trp Lys Glu Phe Lys Pro Val Lys Ser Ser  
 370 375 380  
 Thr Thr Phe Ala Ser Ala Ile Gln Ile Gly Asp Pro Val Ser Ile Asp  
 385 390 395 400

Arg Ala Val His Ala Leu Lys Ser Cys Asp Gly Ile Val Glu Glu Ala  
 405 410 415

Thr Glu Glu Glu Leu Met Asp Ala Thr Ala Gln Ala Asp Ser Thr Gly  
 420 425 430

Met Phe Ile Cys Pro His Thr Gly Val Ala Leu Thr Ala Leu Phe Lys  
 435 440 445

Leu Arg Asn Ser Gly Val Ile Lys Ala Thr Asp Arg Thr Val Val Val  
 450 455 460

Ser Thr Ala His Gly Leu Lys Phe Thr Gln Ser Lys Ile Asp Tyr His  
 465 470 475 480

Ser Lys Asp Ile Lys Asp Met Ala Cys Arg Tyr Ala Asn Pro Pro Met  
 485 490 495

Gln Val Lys Ala Asp Phe Gly Ser Val Met Asp Val Leu Lys Thr Tyr  
 500 505 510

Leu Gln Ser Lys Ala His  
 515

## (2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 643 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: wr1.pk0085.h2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GCTCATCCAG CCCATCGCCA ACGGCGCCAC GGTGCTCTCG CTTGACACGG ATTTGACGG	60
ATGCATGCGG CTTATCAGGG AGGTGACAGC TGAGCTGCCC ATATACCTCG CAAACTCACT	120
CAACTCGCTT CCGGCTGGAG GGGCAGAAGA CTGCAGCCAT CCGAGATATT GCAACANTCA	180
ATTGGCAGGT GCCCGGACTG GGTCACATCC CAAGGAGGCA ATCTGGGGGA ACATTTTATG	240
CTTTCCTACA AGGATTTNAA TTTCCGTGTC CTTNGCTAGT TGATTNCCTT CCNACTCCTT	300
GTTANTNCAA NAGGCCGCCA ACGCAAACCC ACTGTACCCG TACTACAATC CTGGGGTGAC	360
TGATTTCCAT CCACTTGNTT GCCGGGACAA TTTNCATCCN GCAACAATTT GGGGATTCCA	420
TATCNATTAC CNTCGGTTTT TTCNCCCTNA AAGGACNNAT GATTNTCCNA GGAACTCCNN	480
AGGNGGATCA AGGATCCAAA GGCTTTCTAC TCACTGGAAN TTGCTTCCCA ANACGGGGTT	540
CACTNCCGCC CGTTAAACCC NTGACAAGTA TAATGGACAA CACNCCGGGG TNTATNACAA	600
CGGCAANTTN AAANCAAGTT NATCATTAGA ACNGGAANTT NCC	643

## (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 84 amino acids  
 (B) TYPE: amino acid

(C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: wrl.pk0085.h2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```

Leu Ile Gln Pro Ile Ala Asn Gly Ala Thr Val Leu Ser Leu Asp Thr
1           5           10           15
Asp Phe Asp Gly Cys Met Arg Leu Ile Arg Glu Val Thr Ala Glu Leu
20           25           30
Pro Ile Tyr Leu Ala Asn Ser Leu Asn Ser Leu Xaa Leu Glu Gly Gln
35           40           45
Lys Thr Ala Ala Ile Arg Asp Ile Ala Thr Xaa Asn Trp Gln Val Pro
50           55           60
Gly Leu Gly His Ile Pro Arg Arg Gln Ser Xaa Thr Phe Tyr Ala Phe
65           70           75           80
Leu Gln Gly Phe
  
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 525 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```

Leu Ser Ser Cys Leu Phe Asn Ala Ser Val Ser Ser Leu Asn Pro Lys
1           5           10           15
Gln Asp Pro Ile Arg Arg His Arg Ser Thr Ser Leu Leu Arg His Arg
20           25           30
Pro Val Val Ile Ser Cys Thr Ala Asp Gly Asn Asn Ile Lys Ala Pro
35           40           45
Ile Glu Thr Ala Val Lys Pro Pro His Arg Thr Glu Asp Asn Ile Arg
50           55           60
Asp Glu Ala Arg Arg Asn Arg Ser Asn Ala Val Asn Pro Phe Ser Ala
65           70           75           80
Lys Tyr Val Pro Phe Asn Ala Ala Pro Gly Ser Thr Glu Ser Tyr Ser
85           90           95
Leu Asp Glu Ile Val Tyr Arg Ser Arg Ser Gly Gly Leu Leu Asp Val
100          105          110
Glu His Asp Met Glu Ala Leu Lys Arg Phe Asp Gly Ala Tyr Trp Arg
115          120          125
  
```

Asp Leu Phe Asp Ser Arg Val Gly Lys Ser Thr Trp Pro Tyr Gly Ser  
 130 135 140  
 Gly Val Trp Ser Lys Lys Glu Trp Val Leu Pro Glu Ile Asp Asp Asp  
 145 150 155 160  
 Asp Ile Val Ser Ala Phe Glu Gly Asn Ser Asn Leu Phe Trp Ala Glu  
 165 170 175  
 Arg Phe Gly Lys Gln Phe Leu Gly Met Asn Asp Leu Trp Val Lys His  
 180 185 190  
 Cys Gly Ile Ser His Thr Gly Ser Phe Lys Asp Leu Gly Met Thr Val  
 195 200 205  
 Leu Val Ser Gln Val Asn Arg Leu Arg Lys Met Lys Arg Pro Val Val  
 210 215 220  
 Gly Val Gly Cys Ala Ser Thr Gly Asp Thr Ser Ala Ala Leu Ser Ala  
 225 230 235 240  
 Tyr Cys Ala Ser Ala Gly Ile Pro Ser Ile Val Phe Leu Pro Ala Asn  
 245 250 255  
 Lys Ile Ser Met Ala Gln Leu Val Gln Pro Ile Ala Asn Gly Ala Phe  
 260 265 270  
 Val Leu Ser Ile Asp Thr Asp Phe Asp Gly Cys Met Lys Leu Ile Arg  
 275 280 285  
 Glu Ile Thr Ala Glu Leu Pro Ile Tyr Leu Ala Asn Ser Leu Asn Ser  
 290 295 300  
 Leu Arg Leu Glu Gly Gln Lys Thr Ala Ala Ile Glu Ile Leu Gln Gln  
 305 310 315 320  
 Phe Asp Trp Gln Val Pro Asp Trp Val Ile Val Pro Gly Gly Asn Leu  
 325 330 335  
 Gly Asn Ile Tyr Ala Phe Tyr Lys Gly Phe Lys Met Cys Gln Glu Leu  
 340 345 350  
 Gly Leu Val Asp Arg Ile Pro Arg Met Val Cys Ala Gln Ala Ala Asn  
 355 360 365  
 Ala Asn Pro Leu Tyr Leu His Tyr Lys Ser Gly Trp Lys Asp Phe Lys  
 370 375 380  
 Pro Met Thr Ala Ser Thr Thr Phe Ala Ser Ala Ile Gln Ile Gly Asp  
 385 390 395 400  
 Pro Val Ser Ile Asp Arg Ala Val Tyr Ala Leu Lys Lys Cys Asn Gly  
 405 410 415  
 Ile Val Glu Glu Ala Thr Glu Glu Glu Leu Met Asp Ala Met Ala Gln  
 420 425 430  
 Ala Asp Ser Thr Gly Met Phe Ile Cys Pro His Thr Gly Val Ala Leu  
 435 440 445  
 Thr Ala Leu Phe Lys Leu Arg Asn Gln Gly Val Ile Ala Pro Thr Asp  
 450 455 460  
 Arg Thr Val Val Val Ser Thr Ala His Gly Leu Lys Phe Thr Gln Ser  
 465 470 475 480

(2) INFORMATION FOR SEQ ID NO:28:

- ```
(i)    SEQUENCE CHARACTERISTICS:
      (A)  LENGTH:  1478 base pairs
      (B)  TYPE:    nucleic acid
      (C)  STRANDEDNESS:  single
      (D)  TOPOLOGY:  linear

(ii)   MOLECULE TYPE:   cDNA

(vii)  IMMEDIATE SOURCE:
      (B)  CLONE:  cen1.pk0064.f4

(xi)   SEQUENCE DESCRIPTION:  SEQ ID NO:28:
```

|            |            |            |            |            |            |      |
|------------|------------|------------|------------|------------|------------|------|
| CAACAGTGGT | CCTTGAGGGG | GACTCATATG | ATGAAGCTCA | GTCAATGCA  | AAATTGCGTT | 60   |
| GCCAGCAGGA | AGGCCGCACA | TTTGTACCTC | CTTTTGACCA | TCCTGATGTC | ATCACTGGAC | 120  |
| AAGGAACTAT | CGGCATGGAA | ATTGTTAGGC | AGCTGCAAGG | TCCACTGCAT | GCAATATTTG | 180  |
| TACCTGTTGG | AGGTGGTGGA | TTAATTGCTG | GAATTGCTGC | CTATGTAATA | CGGGTTCGCC | 240  |
| CAGAGGTGAA | AATAATTGGA | GTGGAACCCT | CAGATGCAAA | TGCAATGGCA | TTATCCTTGT | 300  |
| GTCATGGTAA | GAGGGTCATG | TTGGAGCATG | TTGGTGGGTT | TGCTGATGGT | GTAGCTGTCA | 360  |
| AAGCTGTTGG | GGAAGAAACA | TTTCGCCTGT | GCAGAGAGCT | AGTAGATGGC | ATTGTTATGG | 420  |
| TCAGTCGAGA | TGCTATTTGT | GCTTCAATAA | AGGATATGTT | TGAGGAGAAA | AGAAGTATCC | 480  |
| TTGAACCTGC | TGGTGCCCTT | GCATTGGCTG | GGGCTGAAGC | CTACTGCAAA | TACTATAACT | 540  |
| TGAAAGGAGA | AACTGTGGTT | GCAATAACTA | GTGGGGCAAA | TATGAACTTT | GATCGACTTA | 600  |
| GACTAGTAAC | CGAGCTAGCT | GATGTTGGCC | GAAAACGGGA | AGCAGTGTTA | GCTACATTTT | 660  |
| TGCCAGAGCG | GCAGGGAAGC | TTCAAAAAAT | TCACAGAATT | GGTTGGCAGG | ATGAATATTA | 720  |
| CTGAATTCAA | ATACAGATAC | GATTCTAATG | CAAAGATGC  | CCTTGTTCTT | TACAGTGTTG | 780  |
| GCATCTACAC | TGACAATGAG | CTTGAGCAA  | TGATGGATCG | CATGGAATCT | GCGAACTGA  | 840  |
| GGACTGTTAA | CCTTACTGAC | AATGATTTGG | CAAAGGACCA | CCTTAGATAC | TTTATTGGAG | 900  |
| GAAGATCAGA | AATAAAAGAT | GAAGTGGTTT | ACCGGTTTAT | TTTCCCGGAA | AGGCCTGGGG | 960  |
| CCCTTATGAA | ATTTTTGGAC | ACGTTTAGTC | CTCGTTGGAA | CATCAGCCTT | TTCCATTACC | 1020 |
| GTGCACAGGG | TGAAGCTGGA | GCAAATGTAT | TAGTTGGTAT | ACAAGTGGCG | CCAGCAGAAT | 1080 |
| TTGATGAATT | CAAGAGTCAT | GCCAACAATC | TTGGGTACGA | GTACATGTCA | GAGCACAACA | 1140 |
| ATGAGATATA | CCGGTTGCTG | TTGCGTGACC | CAAAGGTCTA | ATGTATATGC | CTTTGCTCCC | 1200 |
| ATAATAAGTT | GGTGACACTT | TTCAAGGAAG | ATTTTGCTCC | AAGGTAGAAG | TTGCGAGTTT | 1260 |

CTTCAAGTTG AAATGAAGCC ATCACCAAT GTAGCTTCGG TGTGCCATCT GTTTACTCAG 1320  
 TTAGATCATG TAGTGTATCA GTTGTGTATC TTTGTTGTTG TGCTTCGTGA TCTCAATTTA 1380  
 TTGCTTTGTG CACCTAGAGG TTGTCAAATA ATGATAACCG ATATGTTATC TAAATATCTA 1440  
 ATAATGATTA TGTGATTGTG ATTAAAAAGG GGGGGCCC 1478

## (2) INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 392 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: cen1.pk0064.f4

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Val | Val | Leu | Glu | Gly | Asp | Ser | Tyr | Asp | Glu | Ala | Gln | Ser | Tyr | Ala | 1   | 5   | 10  | 15  |
| Lys | Leu | Arg | Cys | Gln | Gln | Glu | Gly | Arg | Thr | Phe | Val | Pro | Pro | Phe | Asp | 20  | 25  | 30  |     |
| His | Pro | Asp | Val | Ile | Thr | Gly | Gln | Gly | Thr | Ile | Gly | Met | Glu | Ile | Val | 35  | 40  | 45  |     |
| Arg | Gln | Leu | Gln | Gly | Pro | Leu | His | Ala | Ile | Phe | Val | Pro | Val | Gly | Gly | 50  | 55  | 60  |     |
| Gly | Gly | Leu | Ile | Ala | Gly | Ile | Ala | Ala | Tyr | Val | Lys | Arg | Val | Arg | Pro | 65  | 70  | 75  | 80  |
| Glu | Val | Lys | Ile | Ile | Gly | Val | Glu | Pro | Ser | Asp | Ala | Asn | Ala | Met | Ala | 85  | 90  | 95  |     |
| Leu | Ser | Leu | Cys | His | Gly | Lys | Arg | Val | Met | Leu | Glu | His | Val | Gly | Gly | 100 | 105 | 110 |     |
| Phe | Ala | Asp | Gly | Val | Ala | Val | Lys | Ala | Val | Gly | Glu | Glu | Thr | Phe | Arg | 115 | 120 | 125 |     |
| Leu | Cys | Arg | Glu | Leu | Val | Asp | Gly | Ile | Val | Met | Val | Ser | Arg | Asp | Ala | 130 | 135 | 140 |     |
| Ile | Cys | Ala | Ser | Ile | Lys | Asp | Met | Phe | Glu | Glu | Lys | Arg | Ser | Ile | Leu | 145 | 150 | 155 | 160 |
| Glu | Pro | Ala | Gly | Ala | Leu | Ala | Leu | Ala | Gly | Ala | Glu | Ala | Tyr | Cys | Lys | 165 | 170 | 175 |     |
| Tyr | Tyr | Asn | Leu | Lys | Gly | Glu | Thr | Val | Val | Ala | Ile | Thr | Ser | Gly | Ala | 180 | 185 | 190 |     |
| Asn | Met | Asn | Phe | Asp | Arg | Leu | Arg | Leu | Val | Thr | Glu | Leu | Ala | Asp | Val | 195 | 200 | 205 |     |
| Gly | Arg | Lys | Arg | Glu | Ala | Val | Leu | Ala | Thr | Phe | Leu | Pro | Glu | Arg | Gln | 210 | 215 | 220 |     |

Gly Ser Phe Lys Lys Phe Thr Glu Leu Val Gly Arg Met Asn Ile Thr  
 225 230 235 240  
 Glu Phe Lys Tyr Arg Tyr Asp Ser Asn Ala Lys Asp Ala Leu Val Leu  
 245 250 255  
 Tyr Ser Val Gly Ile Tyr Thr Asp Asn Glu Leu Gly Ala Met Met Asp  
 260 265 270  
 Arg Met Glu Ser Ala Lys Leu Arg Thr Val Asn Leu Thr Asp Asn Asp  
 275 280 285  
 Leu Ala Lys Asp His Leu Arg Tyr Phe Ile Gly Gly Arg Ser Glu Ile  
 290 295 300  
 Lys Asp Glu Leu Val Tyr Arg Phe Ile Phe Pro Glu Arg Pro Gly Ala  
 305 310 315 320  
 Leu Met Lys Phe Leu Asp Thr Phe Ser Pro Arg Trp Asn Ile Ser Leu  
 325 330 335  
 Phe His Tyr Arg Ala Gln Gly Glu Ala Gly Ala Asn Val Leu Val Gly  
 340 345 350  
 Ile Gln Val Pro Pro Ala Glu Phe Asp Glu Phe Lys Ser His Ala Asn  
 355 360 365  
 Asn Leu Gly Tyr Glu Tyr Met Ser Glu His Asn Asn Glu Ile Tyr Arg  
 370 375 380  
 Leu Leu Leu Arg Asp Pro Lys Val  
 385 390

## (2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 728 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: sfl1.pk0055.h7

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

|                                                                       |     |
|-----------------------------------------------------------------------|-----|
| AAAATATTGT AGCAATAACC AGTGGAGCAA ACATGAATTT TGATAAACTT CGGGTTGTAA     | 60  |
| CTGAACTTGC TAATGTTGGT CGTAAACAAG AGGCTGTGCT GGCAACTGTT ATGGCAGAGG     | 120 |
| AGCCTGGCAG TTTCAAACAA TTTTGTGAAT TGGTGGGGCA GATGAACATA ACAGAATTCA     | 180 |
| AATACAGATA TAACTCAAAT GAGAAGGCAG TTGTCCTTTA CAGTGTGGG GTTCACACAA      | 240 |
| TCTCCGAACT AAGAGCAATG CAGGAGAGGA TGGAATCTTC TCAGCTCAA ACTTACAATC      | 300 |
| TCACAGAAAG TGA CT TGGTG AAAGACCACT TGC GT TACTT GATGGGAGGC CGATCAAACG | 360 |
| TTCAGAAATGA GGTCTTTGTC GTCTCACCTT TCCAAGAAAG ACTGGTGCTT TGATGAAATT    | 420 |
| TTTGGACCCT TCAGTCCACG TTGGGATATT AGTTTATCCA TTACCGAGGG GAGGTGAAAC     | 480 |
| TGGAGCAAAC TGCTAGTTGG NTACAGGTAC CAAAATGAGA TAGATGAGTC CATGATCGTG     | 540 |



CTAACAAACT GGATATGATT ATAAGTGGNA ATATGTGATG NCTCAGCTCA ATCNCGATGG 600  
 GGNTTAAGCA CTGCATATGG GNATTAGGGG NAGNTACANT TAAATTCACG GCCTCAAGNT 660  
 AAGCATANTN TAGGAACTAG CTTTACAGGG GGCTACNANT TAACCGNGTA TTTTTTTTGA 720  
 GATGANNG 728

## (2) INFORMATION FOR SEQ ID NO:31:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 152 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: sf11.pk0055.h7

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Asn Ile Val Ala Ile Thr Ser Gly Ala Asn Met Asn Phe Asp Lys Leu  
 1 5 10 15  
 Arg Val Val Thr Glu Leu Ala Asn Val Gly Arg Lys Gln Glu Ala Val  
 20 25 30  
 Leu Ala Thr Val Met Ala Glu Glu Pro Gly Ser Phe Lys Gln Phe Cys  
 35 40 45  
 Glu Leu Val Gly Gln Met Asn Ile Thr Glu Phe Lys Tyr Arg Tyr Asn  
 50 55 60  
 Ser Asn Glu Lys Ala Val Val Leu Tyr Ser Val Gly Val His Thr Ile  
 65 70 75 80  
 Ser Glu Leu Arg Ala Met Gln Glu Arg Met Glu Ser Ser Gln Leu Lys  
 85 90 95  
 Thr Tyr Asn Leu Thr Glu Ser Asp Leu Val Lys Asp His Leu Arg Tyr  
 100 105 110  
 Leu Met Gly Gly Arg Ser Asn Val Gln Asn Glu Val Phe Val Val Ser  
 115 120 125  
 Pro Xaa Pro Arg Lys Thr Gly Ala Leu Met Lys Phe Leu Asp Xaa Phe  
 130 135 140  
 Ser Pro Arg Trp Asp Ile Ser Leu  
 145 150

## (2) INFORMATION FOR SEQ ID NO:32:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 572 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: sre.pk0044.f3

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```

AAAGACCTGG TGCTTTGATG AAATTTTGG ACCCCTTCAG TCCACGTTGG AATATCAGTT      60
TATTCCATTA CCGAGGGGAG GGTGAAACTG GAGCAAATGT GCTAGTTGGA ATACAGGTAC      120
CCAAAAGTGA GATGGATGAG TTCCACGATC GTGCCAACAA ACTTGGATAT GATTATAAAG      180
TGGTGAATAA TGATGATGAC TTCCAGCTTC TAATGCACTG ATGATGGTTT TAGGCACTTG      240
CCATTATTGT GTATTTTAGT CAACAAGTTT GCCATATTTA ATATTTCCAC GGTCGTTTCT      300
AAAAGTTGGA TGGGGAAAAA AGGTGGAAAG GAAGTGGCCT TCAGACATGT CATTAGTTGA      360
TTAGAGGAAC AACTAGTTCT TTTTACCTAA TGCGGCGTCT TATTACATTT TTTATAATCT      420
GTAATTTATG TTTTTTTGTT GTTGTTAACA TTGGAATCTT ATAATGTTGT TGCCTGGTCT      480
TTTGTGTCTG TAATATAAGT GTCTTCAAAA GGTGTGTTGC TAAATTTTCAG CAGCCTAAAA      540
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AA                                     572

```

## (2) INFORMATION FOR SEQ ID NO:33:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 72 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: sre.pk0044.f3

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```

Arg Pro Gly Ala Leu Met Lys Phe Leu Asp Pro Phe Ser Pro Arg Trp
1           5           10           15
Asn Ile Ser Leu Phe His Tyr Arg Gly Glu Gly Glu Thr Gly Ala Asn
20          25          30
Val Leu Val Gly Ile Gln Val Pro Lys Ser Glu Met Asp Glu Phe His
35          40          45
Asp Arg Ala Asn Lys Leu Gly Tyr Asp Tyr Lys Val Val Asn Asn Asp
50          55          60
Asp Asp Phe Gln Leu Leu Met His
65          70

```

## (2) INFORMATION FOR SEQ ID NO:34:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 507 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Burkholderia capacia

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Met Ala Ser His Asp Tyr Leu Lys Lys Ile Leu Thr Ala Arg Val Tyr  
1 5 10 15

Asp Val Ala Phe Glu Thr Glu Leu Glu Pro Ala Arg Asn Leu Ser Ala  
20 25 30

Arg Leu Arg Asn Pro Val Tyr Leu Lys Arg Glu Asp Asn Gln Pro Val  
35 40 45

Phe Ser Phe Lys Leu Arg Gly Ala Tyr Asn Lys Met Ala His Ile Pro  
50 55 60

Ala Asp Ala Leu Ala Arg Gly Val Ile Thr Ala Ser Ala Gly Asn His  
65 70 75 80

Ala Gln Gly Val Ala Phe Ser Ala Ala Arg Met Gly Val Lys Ala Val  
85 90 95

Ile Val Val Pro Val Thr Thr Pro Gln Val Lys Val Asp Ala Val Arg  
100 105 110

Ala His Gly Gly Pro Gly Val Glu Val Ile Gln Ala Gly Glu Ser Tyr  
115 120 125

Ser Asp Ala Tyr Ala His Ala Leu Lys Val Gln Glu Glu Arg Gly Leu  
130 135 140

Thr Phe Val His Pro Phe Asp Asp Pro Tyr Val Ile Ala Gly Gln Gly  
145 150 155 160

Thr Ile Ala Met Glu Ile Leu Arg Gln His Gln Gly Pro Ile His Ala  
165 170 175

Ile Phe Val Pro Ile Gly Gly Gly Gly Leu Ala Ala Gly Val Ala Ala  
180 185 190

Tyr Val Lys Ala Val Arg Pro Glu Ile Lys Val Ile Gly Val Gln Ala  
195 200 205

Glu Asp Ser Cys Ala Met Ala Gln Ser Leu Gln Ala Gly Lys Arg Val  
210 215 220

Glu Leu Ala Glu Val Gly Leu Phe Ala Asp Gly Thr Ala Val Lys Leu  
225 230 235 240

Val Gly Glu Glu Thr Phe Arg Leu Cys Lys Glu Tyr Leu Asp Gly Val  
245 250 255

Val Thr Val Asp Thr Asp Ala Leu Cys Ala Ala Ile Lys Asp Val Phe  
260 265 270

Gln Asp Thr Arg Ser Val Leu Glu Pro Ser Gly Ala Leu Ala Val Ala  
275 280 285

Gly Ala Lys Leu Tyr Ala Glu Arg Glu Gly Ile Glu Asn Gln Thr Leu  
290 295 300

Val Ala Val Thr Ser Gly Ala Asn Met Asn Phe Asp Arg Met Arg Phe  
305 310 315 320

Val Ala Glu Arg Ala Glu Val Gly Glu Ala Arg Glu Ala Val Phe Ala  
325 330 335

Val Thr Ile Pro Glu Glu Arg Gly Ser Phe Lys Arg Phe Cys Ser Leu  
 340 345 350

Val Gly Asp Arg Asn Val Thr Glu Phe Asn Tyr Arg Ile Ala Asp Ala  
 355 360 365

Gln Ser Ala His Ile Phe Val Gly Val Gln Ile Arg Arg Arg Gly Glu  
 370 375 380

Ser Ala Asp Ile Ala Ala Asn Phe Glu Ser His Gly Phe Lys Thr Ala  
 385 390 395 400

Asp Leu Thr His Asp Glu Leu Ser Lys Glu His Ile Arg Tyr Met Val  
 405 410 415

Gly Gly Arg Ser Pro Leu Ala Leu Asp Glu Arg Leu Phe Arg Phe Glu  
 420 425 430

Phe Pro Glu Arg Pro Gly Ala Leu Met Lys Phe Leu Ser Ser Met Ala  
 435 440 445

Pro Asp Trp Asn Ile Ser Leu Phe His Tyr Arg Asn Gln Gly Ala Asp  
 450 455 460

Tyr Ser Ser Ile Leu Val Gly Leu Gln Val Pro Gln Ala Asp His Ala  
 465 470 475 480

Glu Phe Glu Arg Phe Leu Ala Ala Leu Gly Tyr Pro Tyr Val Glu Glu  
 485 490 495

Ser Ala Asn Pro Ala Tyr Arg Leu Phe Leu Ser  
 500 505

## (2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1582 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: cc3.mn0002d2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| ACGAGACGAG TCCCCTCCCC CCACCTCGCC TCACCCAACC GGAACGAACA AGTTACCATC  | 60  |
| TCATCCCAAC CCCGCCTCGA CCGGATCTCG TCGGACTCGG ATCCGCCCGA CCACCCCGCG  | 120 |
| CCGCCGCAGA TCAAAGAAGA TGGCAGCTCT CGACACCTTC CTCTTCACCT CGGAGTCTGT  | 180 |
| GAACGAGGGA CACCCTGACA AGCTCTGCGA CCAGGTCTCA GATGCCGTTC TTGACGCTTG  | 240 |
| CCTTGCTGAG GACCCTGACA GCAAGGTTGC TTGTGAGACC TGCACCAAGA CCAACATGGT  | 300 |
| CATGGTCTTT GGTGAGATCA CCACCAAGGC CAATGTCGAC TACGAGAAGA TTGTCAGGGA  | 360 |
| GACCTGCCGC AACATTGGTT TTGTGTCAAA CGATGTCGGG CTTGACGCTG ACCACTGCAA  | 420 |
| GGTGCTCGTG AACATTGAGC AGCAGTCCCC TGATATTGCT CAGGGTGTGC ATGGCCACTT  | 480 |
| CACCAAGCGC CCCGAGGAGA TTGGAGCTGG TGACCAGGGA CACATGTTTCG GGTATGCGAC | 540 |

```

CGATGAGACC CCTGAGTTGA TGCCCCCTCAG CCATGTCCTT GCCACCAAGC TAGGTGCTCG      600
TCTCACCGAG GTCCGCAAGA ACGGAACCTG CCCCTGGCTC AGGCCTGATG GGAAGACCCA      660
GGTGACAGTC GAGTACCGCA ATGAGGGTGG TGCCATGGTC CCCATCCGTG TCCACACCGT      720
CCTCATCTCC ACCCAGCACG ACGAGACAGT GACCAATGAT GAGATCGCTG CTGACCTGAA      780
GGAGCATGTC ATCAAGCCTA TCATCCCTGA GCAGTACCTT GACGAGAAGA CCATCTTCCA      840
CCTTAACCCA TCCGGCCGCT TTGTCATTGG TGGACCTCAC GGCGATGCTG GCCTCACTGG      900
CCGCAAGATC ATCATTGACA CCTACGGTGG CTGGGGAGCC CATGGCGGTG GCGCTTTCTC      960
CGGCAAGGAC CCAACCAAGG TTGACCGCAG CGGAGCCTAT GTCGCGAGGC AGGCTGCCAA     1020
GAGCATCGTC GCCAGCGGCC TTGCTCGCCG CGCCATCGTC CAGGTGTCCT ACGCCATCGG     1080
CGTGCCCGAG CCTCTCTCCG TGTTTGTGCA CACGTACGGC ACCGGCGCGA TCCCCGACAA     1140
GGAGATCCTC AAGATTGTCA AGGAGAACTT CGATTTTCAGG CCTGGCATGA TTATCATCAA     1200
CCTTGACCTC AAGAAAGGCG GCAACGGGCG CTACCTCAAG ACGGCAGCCT ACGGCCACTT     1260
CGGAAGGGAC GACCCTGACT TCACCTGGGA GGTGGTGAAG CCACTCAAGT CGGAGAAACC     1320
TTCTGCCTAA GGCGGCCTTT TTTTCAGTAA GAAGCTTTTG GTGGTCTGCT GTGCTTAATC     1380
ATGCTTTTAT ATGGCTTCTA CATGTTGTGG TTCTTTCTTG ATCTGCACCG CGCTTATCGT     1440
TTGTGTTGTA CTGCCCTAAT AAGTGGTGCT TATGAGGACT GTTTCTGGTT TTGCTGCTTA     1500
TGTTGTAATG CTTTGAAACA ATGAAAGAAG CTACAGGCCA CAGCTATTTT GAGAAGTAAT     1560
GGAACCTCGT GCCGTTTTGA TT   1582

```

## (2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 396 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: cc3.mn0002.d2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```

Met Ala Ala Leu Asp Thr Phe Leu Phe Thr Ser Glu Ser Val Asn Glu
1           5           10           15
Gly His Pro Asp Lys Leu Cys Asp Gln Val Ser Asp Ala Val Leu Asp
20          25          30
Ala Cys Leu Ala Glu Asp Pro Asp Ser Lys Val Ala Cys Glu Thr Cys
35          40          45
Thr Lys Thr Asn Met Val Met Val Phe Gly Glu Ile Thr Thr Lys Ala
50          55          60
Asn Val Asp Tyr Glu Lys Ile Val Arg Glu Thr Cys Arg Asn Ile Gly
65          70          75          80

```

Phe Val Ser Asn Asp Val Gly Leu Asp Ala Asp His Cys Lys Val Leu  
 85 90 95  
 Val Asn Ile Glu Gln Gln Ser Pro Asp Ile Ala Gln Gly Val His Gly  
 100 105 110  
 His Phe Thr Lys Arg Pro Glu Glu Ile Gly Ala Gly Asp Gln Gly His  
 115 120 125  
 Met Phe Gly Tyr Ala Thr Asp Glu Thr Pro Glu Leu Met Pro Leu Ser  
 130 135 140  
 His Val Leu Ala Thr Lys Leu Gly Ala Arg Leu Thr Glu Val Arg Lys  
 145 150 155 160  
 Asn Gly Thr Cys Pro Trp Leu Arg Pro Asp Gly Lys Thr Gln Val Thr  
 165 170 175  
 Val Glu Tyr Arg Asn Glu Gly Gly Ala Met Val Pro Ile Arg Val His  
 180 185 190  
 Thr Val Leu Ile Ser Thr Gln His Asp Glu Thr Val Thr Asn Asp Glu  
 195 200 205  
 Ile Ala Ala Asp Leu Lys Glu His Val Ile Lys Pro Ile Ile Pro Glu  
 210 215 220  
 Gln Tyr Leu Asp Glu Lys Thr Ile Phe His Leu Asn Pro Ser Gly Arg  
 225 230 235 240  
 Phe Val Ile Gly Gly Pro His Gly Asp Ala Gly Leu Thr Gly Arg Lys  
 245 250 255  
 Ile Ile Ile Asp Thr Tyr Gly Gly Trp Gly Ala His Gly Gly Gly Ala  
 260 265 270  
 Phe Ser Gly Lys Asp Pro Thr Lys Val Asp Arg Ser Gly Ala Tyr Val  
 275 280 285  
 Ala Arg Gln Ala Ala Lys Ser Ile Val Ala Ser Gly Leu Ala Arg Arg  
 290 295 300  
 Ala Ile Val Gln Val Ser Tyr Ala Ile Gly Val Pro Glu Pro Leu Ser  
 305 310 315 320  
 Val Phe Val Asp Thr Tyr Gly Thr Gly Ala Ile Pro Asp Lys Glu Ile  
 325 330 335  
 Leu Lys Ile Val Lys Glu Asn Phe Asp Phe Arg Pro Gly Met Ile Ile  
 340 345 350  
 Ile Asn Leu Asp Leu Lys Lys Gly Gly Asn Gly Arg Tyr Leu Lys Thr  
 355 360 365  
 Ala Ala Tyr Gly His Phe Gly Arg Asp Asp Pro Asp Phe Thr Trp Glu  
 370 375 380  
 Val Val Lys Pro Leu Lys Ser Glu Lys Pro Ser Ala  
 385 390 395

## (2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2183 base pairs  
 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: *Oryza sativa*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

|                                                                   |      |
|-------------------------------------------------------------------|------|
| GAATTCTTAT AAATGAACGG AAAATGGAAA AAAAAATTGA TTGGTGCCAC TTCAAAGTTA | 60   |
| AATATGCCAA GACGAATTGA TATGTTTCTG CTGTTGTTTT ATGCTCTTGA TTAGTTGATG | 120  |
| CGCATGTTCA ATGATTTATG ATGTTTGTCT TTGTGGAAAG ATTACATGTA AAGAGTATAG | 180  |
| TAGAACCCTT AAAAGCTAGC CAGCGATTC GCTCTTTTTT TCCAGGTCTC CATGATATGT  | 240  |
| TTACCCTTAA AAGTGGTATA TTTATGTGAT AGTTACAATA CATAGTGGAC CACGATTGAT | 300  |
| TATGCGTTTA TGCTGATTCC GGCAGAAAAT TGTTAGATTC CTTGTGCTCT ATACCTGCTT | 360  |
| GTTGCGCTTG TAGAGAATAT TACAAATACC TAACACTTGC CCAAGGAACT TAGGAACTTA | 420  |
| GTCAACTCTT TGTAGGGACA ACTATTTTAG CCCAAAATTG TGGTCTTGTC AGGTGCCAAC | 480  |
| AAAACAGCAT CTGGCGTAC ATAAGCTATA TAGAGGATTA AAAGGAATGT TTTGTTCCCTT | 540  |
| GCTACTGTTT TTTTAACCTG TTTACTCAGG ACAAATTTTG TTGCATAAAC CATTTGTTCT | 600  |
| AGGGATCAGT ATTGTCCTCT CAGTGTGTTA TGTAAGCATT TCCAGAAATC AATTGTCGCT | 660  |
| ATCAGCTTCC CTCACATTAG CTATCACTTA TACCCCTTTT TTTCTCATAG GCTCACCATG | 720  |
| TCCATTTTAT TCATGATATT TCTTTGTCTA AAGTATGTGA AATACCATTT TATGCAGATA | 780  |
| GGAGAAGATG GCCGCACTTG ATACCTTCCT CTTTACCTCG GAGTCTGTGA ACGAGGGCCA | 840  |
| CCCTGACAAG CTCTGCGACC AAGTCTCAGA TGCTGTGCTT GATGCCTGCC TCGCCGAGGA | 900  |
| CCCTGACAGC AAGGTGCTT GTGAGACCTG CACCAAGACA AACATGGTCA TGGTCTTTGG  | 960  |
| TGAGATCACC ACCAAGGCTA ACGTTGACTA TGAGAAGATT GTCAGGGAGA CATGCCGTAA | 1020 |
| CATCGGTTTT GTGTCAGCTG ATGTCGGTCT CGATGCTGAC CACTGCAAGG TGCTTGTGAA | 1080 |
| CATCGAGCAG CAGTCCCTG ACATTGCACA GGGTGTGCAC GGGCACTTCA CCAAGCGCCC  | 1140 |
| TGAGGAGATT GGTGCTGGTG ACCAGGGACA CATGTTTGA TATGCAACTG ATGAGACCCC  | 1200 |
| TGAGTTGATG CCCCTCAGCC ATGTCCTTGC TACCAAGCTT GCGCTCGTC TTACGGAGGT  | 1260 |
| TCGCAAGAAT GGGACCTGCG CATGGCTCAG GCCTGACGGG AAGACCCAAG TGACTGTTGA | 1320 |
| GTACCGCAAT GAGAGCGGTG CCAGGGTCCC TGTCCGTGTC CACACCGTCC TCATCTCTAC | 1380 |
| CCAGCATGAT GAGACAGTCA CCAACGATGA GATTGCTGCT GACCTGAAGG AGCATGTCAT | 1440 |
| CAAGCCTGTC ATTCCCGAGC AGTACCTTGA TGAGAAGACA ATCTTCCATC TTAACCCATC | 1500 |
| TGGTCGCTTC GTCATTGGCG GACCTCATGG TGATGCTGGT CTCACTGGCC GGAAGATCAT | 1560 |
| CATTGACACT TATGGTGGCT GGGGAGCTCA CGGTGGTGGT GCCTTCTCTG GCAAGGACCC | 1620 |
| AACCAAGGTT GACCGCAGTG GAGCATACGT CGCAAGGCAA GCTGCCAAGA GCATTGTTGC | 1680 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| TAGTGGCCTT GCTCGCCGCT GCATTGTCCA AGTATCATAC GCCATCGGTG TCCCAGAGCC | 1740 |
| ACTGTCCGTA TTCGTCGACA CATACGGCAC TGGCAGGATC CCTGACAAGG AGATCCTCAA | 1800 |
| GATTGTGAAG GAGAACTTCG ACTTCAGGCC TGGCATGATC ATCATCAACC TTGACCTCAA | 1860 |
| GAAAGGCGGC AACGGACGCT ACCTCAAGAC GGCGGCTTAC GGTCACCTCG GAAGGGACGA | 1920 |
| CCCAGACTTC ACCTGGGAGG TGGTGAAGCC CCTCAAGTGG GAGAAGCCTT CTGCCTAAAA | 1980 |
| GCTCCCTTTC GGAGGCTTTT GCTCTGTCCC ATTATGGTGT TTTGTTTCCT CGCTGCTCAG | 2040 |
| CATTGTGATT CTTAACCTGC CCCCCGCTGC CATTTATGCC CATGCACGCT ACTTTCCTAA | 2100 |
| TAATAAGTAC TTATAAGGGT ATTGTGTTTG AATATTTTAC CTAGAGGAGG AGGAGGATTT | 2160 |
| GTTATCTGTT ATTGCTTAAG CTT                                         | 2183 |

## (2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1485 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: s2.12b06
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

|                                                                   |      |
|-------------------------------------------------------------------|------|
| AGCCAAGCCC CACTCAACCA CCACACCACT CTCTCTGCTC TTCTTCTACC TTTCAAGTTT | 60   |
| TTAAAGTATT AAGATGGCAG AGACATTCTT ATTTACCTCA GAGTCAGTGA ACGAGGGACA | 120  |
| CCCTGACAAG CTCTGCGACC AAATCTCCGA TGCTGTCTCT GACGCTTGCC TTGAACAGGA | 180  |
| CCCAGACAGC AAGGTTGCCT GCGAAACATG CACCAAGACC AACTTGGTCA TGGTCTTCGG | 240  |
| AGAGATCACC ACCAAGGCCA ACGTTGACTA CGAGAAGATC GTGCGTGACA CCTGCAGGAA | 300  |
| CATCGGCTTC GTCTCAAACG ATGTGGGACT TGATGCTGAC AACTGCAAGG TCCTTGTAAG | 360  |
| CATTGAGCAG CAGAGCCCTG ATATTGCCCA GGGTGTGCAC GGCCACCTTA CCAAAGACC  | 420  |
| CGAGGAAATC GGTGCTGGAG ACCAGGGTCA CATGTTTGGC TATGCCACGG ACGAAACCCC | 480  |
| AGAATTGATG CCATTGAGTC ATGTTCTTGC AACTAACTC GGTGCTCGTC TCACCGAGGT  | 540  |
| TCGCAAGAAC GGAACCTGCC CATGGTTGAG GCCTGATGGG AAAACCCAAG TGAAGTTGA  | 600  |
| GTATTACAAT GACAACGGTG CCATGGTTCC AGTTCGTGTC CACACTGTGC TTATCTCCAC | 660  |
| CCAACATGAT GAGACTGTGA CCAACGACGA AATTGCAGCT GACCTCAAGG AGCATGTGAT | 720  |
| CAAGCCGGTG ATCCCGGAGA AGTACCTTGA TGAGAAGACC ATTTTCCACT TGAACCCCTC | 780  |
| TGGCCGTTTT GTCATTGGAG GTCCTCACGG TGATGCTGGT CTCACCGGCC GCAAGATCAT | 840  |
| CATCGATACT TACGGAGGAT GGGGTGCTCA TGGTGGTGGT GCTTTCTCCG GGAAGGATCC | 900  |
| CACCAAGGTT GATAGGAGTG GTGCTTACAT TGTGAGACAG GCTGCTAAGA GCATTGTGGC | 960  |
| AAGTGGACTA GCCAGAAGGT GCATTGTGCA AGTGTCTTAT GCCATTGGTG TGCCCAGGCC | 1020 |



TTTGTCTGTC TTTGTTGACA CCTATGGCAC CGGGAAGATC CATGATAAGG AGATTCTCAA 1080  
 CATTGTGAAG GAGAACTTTG ATTTCAAGGCC CGGTATGATC TCCATCAACC TTGATCTCAA 1140  
 GAGGGGTGGG AATAACAGGT TCTTGAAGAC TGCTGCATAT GGACACTTCG GCAGAGAGGA 1200  
 CCCTGACTTC ACATGGGAAG TGGTCAAGCC CCTCAAGTGG GAGAAGGCCT AAGGCCATTC 1260  
 ATTCCACTGC AATGTGCTGG GAGTTTTTTA GCGTTGCCCT TATAATGTCT ATTATCCATA 1320  
 ACTTTCCACG TCCCTTGCTC TGTGTTTTTC TCTCGTCGTC CTCCTCCTAT TTTGTTTCTC 1380  
 CTGCCTTTCA TTTGTAATTT TTTACATGAT CAACTAAAAA ATGTACTCTC TGTTTTCCGA 1440  
 CCATTGTGTC TCTTAATATC AGTATCAAAA AGAATGTTCC AAGTT 1485

## (2) INFORMATION FOR SEQ ID NO:39:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 392 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: not relevant  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: s2.12b06

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Ala Glu Thr Phe Leu Phe Thr Ser Glu Ser Val Asn Glu Gly His  
 1 5 10 15  
 Pro Asp Lys Leu Cys Asp Gln Ile Ser Asp Ala Val Leu Asp Ala Cys  
 20 25 30  
 Leu Glu Gln Asp Pro Asp Ser Lys Val Ala Cys Glu Thr Cys Thr Lys  
 35 40 45  
 Thr Asn Leu Val Met Val Phe Gly Glu Ile Thr Thr Lys Ala Asn Val  
 50 55 60  
 Asp Tyr Glu Lys Ile Val Arg Asp Thr Cys Arg Asn Ile Gly Phe Val  
 65 70 75 80  
 Ser Asn Asp Val Gly Leu Asp Ala Asp Asn Cys Lys Val Leu Val Asn  
 85 90 95  
 Ile Glu Gln Gln Ser Pro Asp Ile Ala Gln Gly Val His Gly His Leu  
 100 105 110  
 Thr Lys Arg Pro Glu Glu Ile Gly Ala Gly Asp Gln Gly His Met Phe  
 115 120 125  
 Gly Tyr Ala Thr Asp Glu Thr Pro Glu Leu Met Pro Leu Ser His Val  
 130 135 140  
 Leu Ala Thr Lys Leu Gly Ala Arg Leu Thr Glu Val Arg Lys Asn Gly  
 145 150 155 160  
 Thr Cys Pro Trp Leu Arg Pro Asp Gly Lys Thr Gln Val Thr Val Glu  
 165 170 175

Tyr Tyr Asn Asp Asn Gly Ala Met Val Pro Val Arg Val His Thr Val  
 180 185 190  
 Leu Ile Ser Thr Gln His Asp Glu Thr Val Thr Asn Asp Glu Ile Ala  
 195 200 205  
 Ala Asp Leu Lys Glu His Val Ile Lys Pro Val Ile Pro Glu Lys Tyr  
 210 215 220  
 Leu Asp Glu Lys Thr Ile Phe His Leu Asn Pro Ser Gly Arg Phe Val  
 225 230 235 240  
 Ile Gly Gly Pro His Gly Asp Ala Gly Leu Thr Gly Arg Lys Ile Ile  
 245 250 255  
 Ile Asp Thr Tyr Gly Gly Trp Gly Ala His Gly Gly Gly Ala Phe Ser  
 260 265 270  
 Gly Lys Asp Pro Thr Lys Val Asp Arg Ser Gly Ala Tyr Ile Val Arg  
 275 280 285  
 Gln Ala Ala Lys Ser Ile Val Ala Ser Gly Leu Ala Arg Arg Cys Ile  
 290 295 300  
 Val Gln Val Ser Tyr Ala Ile Gly Val Pro Glu Pro Leu Ser Val Phe  
 305 310 315 320  
 Val Asp Thr Tyr Gly Thr Gly Lys Ile His Asp Lys Glu Ile Leu Asn  
 325 330 335  
 Ile Val Lys Glu Asn Phe Asp Phe Arg Pro Gly Met Ile Ser Ile Asn  
 340 345 350  
 Leu Asp Leu Lys Arg Gly Gly Asn Asn Arg Phe Leu Lys Thr Ala Ala  
 355 360 365  
 Tyr Gly His Phe Gly Arg Glu Asp Pro Asp Phe Thr Trp Glu Val Val  
 370 375 380  
 Lys Pro Leu Lys Trp Glu Lys Ala  
 385 390

## (2) INFORMATION FOR SEQ ID NO:40:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1479 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Lycopersicon esculentum*

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| GAATTCCTAC AAAGAGGTTA TTTCTCTCAA GGGGTAAAAA GATTGCCCTT TTTCGACATT | 60  |
| TATAATCCTC TTTTCTCTT TGTTCCCGT TGGGTTCTT ACTTTCCTGT TTCTTGAGAA    | 120 |
| TGGAACTTT CTTATTCACC TCCGAGTCTG TGAACGAGGG TCACCCAGAC AAGCTCTGTG  | 180 |
| ATCAGATCTC TGATGCAGTT CTTGATGCCT GCCTTGAGCA AGATCCCGAG AGCAAAGTTG | 240 |
| CATGTGAAAC TTGCACCAAG ACCAACTTGG TCATGGTCTT TGGTGAGATC ACAACCAAGG | 300 |

|                                                                   |      |
|-------------------------------------------------------------------|------|
| CTATTGTAGA CTATGAGAAG ATTGTGCGTG ACACATGCCG TAATATTGGA TTTGTTTCTG | 360  |
| ATGATGTTGG TCTTGATGCT GACAACTGCA AGGTCCTTGT TTACATTGAG CAGCAAAGTC | 420  |
| CTGATATTGC TCAAGGTGTC CACGGCCATC TGACCAAACG CCCCAGGAG ATTGGTGCTG  | 480  |
| GTGACCAGGG CCACATGTTT GGCTATGCAA CAGATGAGAC CCCTGAATTA ATGCCTCTCA | 540  |
| GTCACGTGCT TGCAACTAAA CTTGGTGCCC GTCTTACAGA AGTCCGCAAG AATGGCACCT | 600  |
| GCGCCTGGTT GAGGCCTGAT GGCAAGACCC AAGTTACTGT TGAGTATAGC AATGACAATG | 660  |
| GTGCCATGGT TCCAATTAGG GTACACACTG TTCTTATCTC CACCCAACAC GATGAGACCG | 720  |
| TTACCAATGA TGAGATTGCC CGCGACCTTA AGGAGCATGT CATCAAACCA GTCATCCCAG | 780  |
| AGAAGTACCT TGATGAGAAT ACTATTTTCC ACCTTAACCC ATCTGGCCGA TTCGTTATTG | 840  |
| GTGGACCTCA TGGTGATGCT GGTCTCACTG GTCGTAAAAT CATCATCGAC ACTTATGGTG | 900  |
| GTTGGGGTGC TCATGGTGGT GGTGCTTTCT CGGGCAAAGA CCCAACCAAG GTCGACAGGA | 960  |
| GTGGTGCATA CATTGTAAGG CAGGCTGCAA AGAGTATCGT AGCTAGTGGA CTTGCTCGTA | 1020 |
| GATGCATCGT GCAGGTATCT TATGCCATCG GTGTGCCTGA GCCATTGTCT GTATTGCTTG | 1080 |
| ACACCTATGG CACTGGAAAG ATCCCTGACA GGGAAATTTT GAAGATCGTT AAGGAGAACT | 1140 |
| TTGACTTCAG ACCTGGAATG ATGTCCATTA ACTTGATTTT GAAGAGGGGT GGCAATAGAA | 1200 |
| GATTCTTGAA AACTGCTGCC TATGGTCACT TTGGACGTGA TGACCCCGAT TTCACATGGG | 1260 |
| AAGTTGTCAA GCCCCTCAAG TGGGAAAAGC CCCAAGACTA ATAAGTGCTT GCCTATGTTT | 1320 |
| TTGTTCTTTG TTGTTTGCTT GTGGCTTTAG AATCTCCCCC GTGTTTGCTT GTTTGTCTTT | 1380 |
| GTATTTTCTC TTTTGACCCT TTATTTTGTT ATTGTCCTGT TTCCATTGTG TTGGATGGAT | 1440 |
| ATCTTAGGCC TTGGAATATT AAGGAAAGAA AAGGAATTC                        | 1479 |

## (2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1380 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

|                                                                   |     |
|-------------------------------------------------------------------|-----|
| CCCTCCCTTC GGTTCATCGG CCTCCCGATC GAGCAGTAGA AGCAGCGCAA GGGCATCGCT | 60  |
| AGCACTAAAG AAATGGCAGC CGAGACGTTT CTCTTCACGT CCGAGTCTGT GAACGAGGGC | 120 |
| CATCCCGACA AGCTCTGTGA CCAAGTCTCC GACGCCGTCT TGGATGCCTG CTTGGCCCAG | 180 |
| GATGCCGACA GCAAGGTCGC CTGCGAGACC GTCACCAAGA CCAACATGGT CATGGTCTTG | 240 |
| GGCGAGATCA CCACCAAGGC CACCGTCGAC TATGAGAAGA TCGTGCGTGA CACCTGCCGC | 300 |
| AACATCGGTT TCATCTCTGA TGACGTTGGT CTCGACGCCG ACCGTTGCAA RGTGCTCGTC | 360 |
| AACATCGAGC AGCAGTCCCC TGACATTGCC CAGGGTGTTT ATGGACACTT CACCAAGCGT | 420 |

```

CCCGAAGAAG TCGGCGCCGG TGACCAGGGC ATCATGTTCTG GCTATGCCAC CGATGAGACC      480
CCTGAGCTGA TGCCCCTCAA GCACGTGCTT GCCACCAAGC TYGGAGCTCG CCTCACSGAG      540
GTCCGCAAGA ATGGCACCTG CGCCTGGGTC AGGCCTGACG GAAAGACCCA GGTCACAGTC      600
GAGTACCTAA ACGAGGATGG TGCCATGGTA CCTGTTCGTG TGCACACCGT CCTCATCTCC      660
ACCCAGCACG ACGAGACCGT CACCAACGAC GAGATTGCTG CGGACCTCAA GGAGCATGTC      720
ATCAAGCCGG TGATCCCCGC AAAGTACCTC GATGAGAACA CCATCTTCCA CCTGAACCCG      780
TCTGGCCGCT TCGTCATCGG CGGCCCCAC GGTGACGCCG GTCTCACC GGCAAGATC      840
ATCATCGACA CCTATGGTGG CTGGGGAGCC CACGGCGGCG GTGCCTTCTC TGGCAAGGAC      900
CCAACCAAGG TCGACCGYAG TGGCGCCTAC ATTGCCAGGC ARGCCGCCAA GAGCATCATC      960
GCCAGCGGCC TCGCACGCCG CTGCATTGTG CAGATCTCAT ACGCCATCGG TGTGCCTGAG     1020
CCTTTGTCTG TGTTCTGTCG CTCCTACGGC ACCGGCAAGA TCCCCGACAG GGAGATCCTC     1080
AAGCTCGTGA AGGAGAACTT TGA CTTCAGG CCCGGGATGA TCAGCATCAA CCTGGACTTG     1140
AAGAAAGGTG GAAACAGGTT CATCAAGACC GCTGCTTACG GTCACCTTGG CCGTGATGAT     1200
GCCGACTTCA CCTGGGAGGT GGTGAAGCCC CTCAAGTTCG ACAAGGCATC TGCCTAAGAG     1260
CATGGCATTC TCTTGGTCTG CCGCCTCTCA AGTTCGTCAA GACGGGATCA TGTTGCTCCT     1320
GGGAAGTGGG AAGAAGCATT AGACATTGAA GCGACGCTCT ACACTGGTCT TGTTGTATGG     1380

```

## (2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 394 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: not relevant
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

```

Met Ala Ala Glu Thr Phe Leu Phe Thr Ser Glu Ser Val Asn Glu Gly
1           5           10           15
His Pro Asp Lys Leu Cys Asp Gln Val Ser Asp Ala Val Leu Asp Ala
20          25          30
Cys Leu Ala Gln Asp Ala Asp Ser Lys Val Ala Cys Glu Thr Val Thr
35          40          45
Lys Thr Asn Met Val Met Val Leu Gly Glu Ile Thr Thr Lys Ala Thr
50          55          60
Val Asp Tyr Glu Lys Ile Val Arg Asp Thr Cys Arg Asn Ile Gly Phe
65          70          75          80
Ile Ser Asp Asp Val Gly Leu Asp Ala Asp Arg Cys Lys Val Leu Val
85          90          95
Asn Ile Glu Gln Gln Ser Pro Asp Ile Ala Gln Gly Val His Gly His
100         105         110

```

Phe Thr Lys Arg Pro Glu Glu Val Gly Ala Gly Asp Gln Gly Ile Met  
 115 120 125  
 Phe Gly Tyr Ala Thr Asp Glu Thr Pro Glu Leu Met Pro Leu Lys His  
 130 135 140  
 Val Leu Ala Thr Lys Leu Gly Ala Arg Leu Thr Glu Val Arg Lys Asn  
 145 150 155 160  
 Gly Thr Cys Ala Trp Val Arg Pro Asp Gly Lys Thr Gln Val Thr Val  
 165 170 175  
 Glu Tyr Leu Asn Glu Asp Gly Ala Met Val Pro Val Arg Val His Thr  
 180 185 190  
 Val Leu Ile Ser Thr Gln His Asp Glu Thr Val Thr Asn Asp Glu Ile  
 195 200 205  
 Ala Ala Asp Leu Lys Glu His Val Ile Lys Pro Val Ile Pro Ala Lys  
 210 215 220  
 Tyr Leu Asp Glu Asn Thr Ile Phe His Leu Asn Pro Ser Gly Arg Phe  
 225 230 235 240  
 Val Ile Gly Gly Pro His Gly Asp Ala Gly Leu Thr Gly Arg Lys Ile  
 245 250 255  
 Ile Ile Asp Thr Tyr Gly Gly Trp Gly Ala His Gly Gly Gly Ala Phe  
 260 265 270  
 Ser Gly Lys Asp Pro Thr Lys Val Asp Arg Ser Gly Ala Tyr Ile Ala  
 275 280 285  
 Arg Gln Ala Ala Lys Ser Ile Ile Ala Ser Gly Leu Ala Arg Arg Cys  
 290 295 300  
 Ile Val Gln Ile Ser Tyr Ala Ile Gly Val Pro Glu Pro Leu Ser Val  
 305 310 315 320  
 Phe Val Asp Ser Tyr Gly Thr Gly Lys Ile Pro Asp Arg Glu Ile Leu  
 325 330 335  
 Lys Leu Val Lys Glu Asn Phe Asp Phe Arg Pro Gly Met Ile Ser Ile  
 340 345 350  
 Asn Leu Asp Leu Lys Lys Gly Gly Asn Arg Phe Ile Lys Thr Ala Ala  
 355 360 365  
 Tyr Gly His Phe Gly Arg Asp Asp Ala Asp Phe Thr Trp Glu Val Val  
 370 375 380  
 Lys Pro Leu Lys Phe Asp Lys Ala Ser Ala  
 385 390

## (2) INFORMATION FOR SEQ ID NO:43:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1353 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Hordeum vulgare*

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

|                                                                    |      |
|--------------------------------------------------------------------|------|
| GAATTCCGGA TAGCATCAGC ACAACTGCAC GAGAGCATCT CTACCACCAA AGAAATGGCG  | 60   |
| GCCGAGACGT TCCTCTTCAC GTCCGAGTCC GTGAACGAGG GCCATCCCGA CAAGCTGTGC  | 120  |
| GACCAGGTCT CTGACGCCGT CTTGGACGCC TGCTTGGCCC AGGATCCTGA CAGCAAGGTT  | 180  |
| GCTTGCGAGA CCTGCACCAA GACCAACATG GTCATGGTCT TCGGCGAGAT CACCACCAAG  | 240  |
| GCCACCGTTG ACTATGAGAA GATTGTGCGC GACACCTGCC GTGACATCGG CTTCATCTCT  | 300  |
| GACGACGTCG GTCTCGATGC CGACCATTGC AAGGTGCTCG TCAACATCGA GCAGCAATCC  | 360  |
| CCTGACATTG CCCAGGGTGT TCACGGACAC TTCACCAAGC GTCCAGAAGA GGTCGGCGCC  | 420  |
| GGTGACCAGG GCATCATGTT TGGCTACGCC ACTGATGAGA CCCCTGAGCT GATGCCCCTC  | 480  |
| ACCCACATGC TTGCCACCAA GCTCGGAGCT CGCCTCACCG AGGTCCGCAA GAATGGCACC  | 540  |
| TGCGCCTGGC TCAGGCCTGA TGGAAAGACC CAGGTCACCA TTGAGTACCT AAACGAGGGT  | 600  |
| GGTGCCATGG TGCCCGTTCTG TGTGCACACC GTCCTCATCT CCACCCAGCA TGATGAGACC | 660  |
| GTCACCAACG ATGAGATCGC TGCAGACCTC AAGGAGCATG TCATCAAGCC GGTGATTCCC  | 720  |
| GGGAAGTACC TCGATGAGAA CACCATCTTC CACCTGAACC CATCGGGCCG CTTTGTCTATC | 780  |
| GGTGGCCCTC ACGGCGATGC CGGTCTCACC GCCCGCAAGA TCATCATCGA CACCTATGGT  | 840  |
| GGCTGGGGAG CCCACGGCGG CGGTGCCTTC TCTGGCAAGG ACCCTACCAA GGTCGACCGC  | 900  |
| AGTGGCGCCT ACATTGCCAG GCAGGCTGCC AAGAGCATCA TCGCCAGCGG CCTCGCACGC  | 960  |
| CGGTGCATTG TGCAGATCTC ATATGCCATC GGTGTACCTG AGCCTTTGTC TGTGTTTCGTC | 1020 |
| GACTCCTACG GCACTGGCAA GATCCCTGAC AGGGAGATCC TCAAGCTCGT GAAGGAGAAC  | 1080 |
| TTTGACTTCA GACCCGGGAT GATCACGATC AACCTCGACT TGAAGAAAGG TGGAAACAGG  | 1140 |
| TTCATCAAGA CAGCTGCTTA CGGTCACTTT GGCCGCGATG ATGCTGACTT CACCTGGGAG  | 1200 |
| GTGGTGAAGC CCCTCAAGTT CGACAAGGCA TCTGCTTAAG AAGAAGACAT CACATTGAGG  | 1260 |
| GTTCTTCTTG GTCTGATGCC TCTCAAGTTC GGCAAGGCGG GATCCTTTTG CTCCTCGGAA  | 1320 |
| GTAAGAAGAA GCATTCAACA TCGCCCGGAA TTC                               | 1353 |

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding all or a substantial portion of a plant dihydropicolinate reductase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2 and 4;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2 and 4; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

2. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:1 and 3.

3. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.

4. A transformed host cell comprising the chimeric gene of Claim 3.

5. A dihydropicolinate reductase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2 and 4.

6. An isolated nucleic acid fragment encoding all or a substantial portion of a plant diaminopimelate epimerase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:7, 9, 11, and 13;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:7, 9, 11, and 13; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

7. The isolated nucleic acid fragment of Claim 6 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:6, 8, 10, and 12.

8. A chimeric gene comprising the nucleic acid fragment of Claim 6 operably linked to suitable regulatory sequences.

9. A transformed host cell comprising the chimeric gene of Claim 8.

10. A diaminopimelate epimerase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 7, 9, 11, and 13.

11. An isolated nucleic acid fragment encoding all or a substantial portion of a  
5 plant threonine synthase comprising a member selected from the group consisting of:

(a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:16 and 18;

(b) an isolated nucleic acid fragment that is substantially similar to an  
10 isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:16 and 18; and

(c) an isolated nucleic acid fragment that is complementary to (a) or (b).

12. The isolated nucleic acid fragment of Claim 11 wherein the nucleotide  
15 sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:15 and 17.

13. A chimeric gene comprising the nucleic acid fragment of Claim 11 operably linked to suitable regulatory sequences.

14. A transformed host cell comprising the chimeric gene of Claim 13.

15. A threonine synthase polypeptide comprising all or a substantial portion of the  
20 amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:16 and 18.

16. An isolated nucleic acid fragment encoding all or a substantial portion of a  
25 plant threonine synthase comprising a member selected from the group consisting of:

(a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:20;

(b) an isolated nucleic acid fragment that is substantially similar to an  
isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:20; and

(c) an isolated nucleic acid fragment that is complementary to (a) or (b).  
30

17. The isolated nucleic acid fragment of Claim 16 wherein the nucleotide  
sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID  
NO:19.

18. A chimeric gene comprising the nucleic acid fragment of Claim 16 operably  
35 linked to suitable regulatory sequences.

19. A transformed host cell comprising the chimeric gene of Claim 18.

20. A threonine synthase polypeptide comprising all or a substantial portion of the  
amino acid sequence set forth in SEQ ID NO:20.



21. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine synthase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:22 and 24;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:22 and 24; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

22. The isolated nucleic acid fragment of Claim 21 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:21 and 23.

23. A chimeric gene comprising the nucleic acid fragment of Claim 21 operably linked to suitable regulatory sequences.

24. A transformed host cell comprising the chimeric gene of Claim 23.

25. A threonine synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:22 and 24.

26. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine synthase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:26;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:26; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

27. The isolated nucleic acid fragment of Claim 26 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:25.

28. A chimeric gene comprising the nucleic acid fragment of Claim 26 operably linked to suitable regulatory sequences.

29. A transformed host cell comprising the chimeric gene of Claim 28.

30. A threonine synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:26.

31. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine deaminase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:29;

- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:29; and

- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

5           32. The isolated nucleic acid fragment of Claim 31 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:28.

          33. A chimeric gene comprising the nucleic acid fragment of Claim 31 operably linked to suitable regulatory sequences.

10           34. A transformed host cell comprising the chimeric gene of Claim 33.

          35. A threonine deaminase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:29.

          36. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine deaminase comprising a member selected from the group consisting of:

15           (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:31 and 33;

          (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:31 and 33; and

20           (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

          37. The isolated nucleic acid fragment of Claim 36 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:30 and 32.

25           38. A chimeric gene comprising the nucleic acid fragment of Claim 36 operably linked to suitable regulatory sequences.

          39. A transformed host cell comprising the chimeric gene of Claim 38.

30           40. A threonine deaminase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:31 and 33.

          41. An isolated nucleic acid fragment encoding all or a substantial portion of a plant S-adenosylmethionine synthetase wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:35.

35           42. A chimeric gene comprising the nucleic acid fragment of Claim 41 operably linked to suitable regulatory sequences.

          43. A transformed host cell comprising the chimeric gene of Claim 42.

44. An isolated nucleic acid fragment encoding all or a substantial portion of a plant S-adenosylmethionine synthetase wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:38.

45. A chimeric gene comprising the nucleic acid fragment of Claim 44 operably  
5 linked to suitable regulatory sequences.

46. A transformed host cell comprising the chimeric gene of Claim 45.

47. An isolated nucleic acid fragment encoding all or a substantial portion of a plant S-adenosylmethionine synthetase wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:41.

48. A chimeric gene comprising the nucleic acid fragment of Claim 47 operably  
10 linked to suitable regulatory sequences.

49. A transformed host cell comprising the chimeric gene of Claim 48.

50. A method of altering the level of expression of a plant amino acid biosynthetic enzyme in a host cell comprising:

15 (a) transforming a host cell with the chimeric gene of any of Claims 3, 8, 13, 18, 23, 28, 33, 38, 42, 45, and 48; and  
(b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene  
wherein expression of the chimeric gene results in production of altered levels of a plant  
20 amino acid biosynthetic enzyme in the transformed host cell.

51. A method of obtaining a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a plant amino acid biosynthetic enzyme comprising:

25 (a) probing a cDNA or genomic library with the nucleic acid fragment of any of Claims 1, 6, 11, 16, 21, 26, 31, 36, 41, 44, and 47;  
(b) identifying a DNA clone that hybridizes with the nucleic acid fragment of any of Claims 1, 6, 11, 16, 21, 26, 31, 36, 41, 44, and 47;  
(c) isolating the DNA clone identified in step (b); and  
(d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

30 wherein the sequenced nucleic acid fragment encodes all or substantially all of the amino acid sequence encoding a plant amino acid biosynthetic enzyme.

52. A method of obtaining a nucleic acid fragment encoding a portion of an amino acid sequence encoding a plant amino acid biosynthetic enzyme comprising:

35 (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs:1, 3, 6, 8, 10, 12, 15, 17, 19, 21, 23, 25, 28, 30, 32, 35, 38, and 41; and  
(b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector

wherein the amplified nucleic acid fragment encodes a portion of an amino acid sequence encoding a plant amino acid biosynthetic enzyme.

53. The product of the method of Claim 51.

54. The product of the method of Claim 52.

5 55. A method for evaluating at least one compound for its ability to inhibit the activity of a plant biosynthetic enzyme selected from the group consisting of dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase, the method comprising the steps of:

10 (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a plant biosynthetic enzyme selected from the group consisting of dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase, operably linked to suitable regulatory sequences;

15 (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the biosynthetic enzyme encoded by the operably linked nucleic acid fragment in the transformed host cell;

20 (c) optionally purifying the biosynthetic enzyme expressed by the transformed host cell;

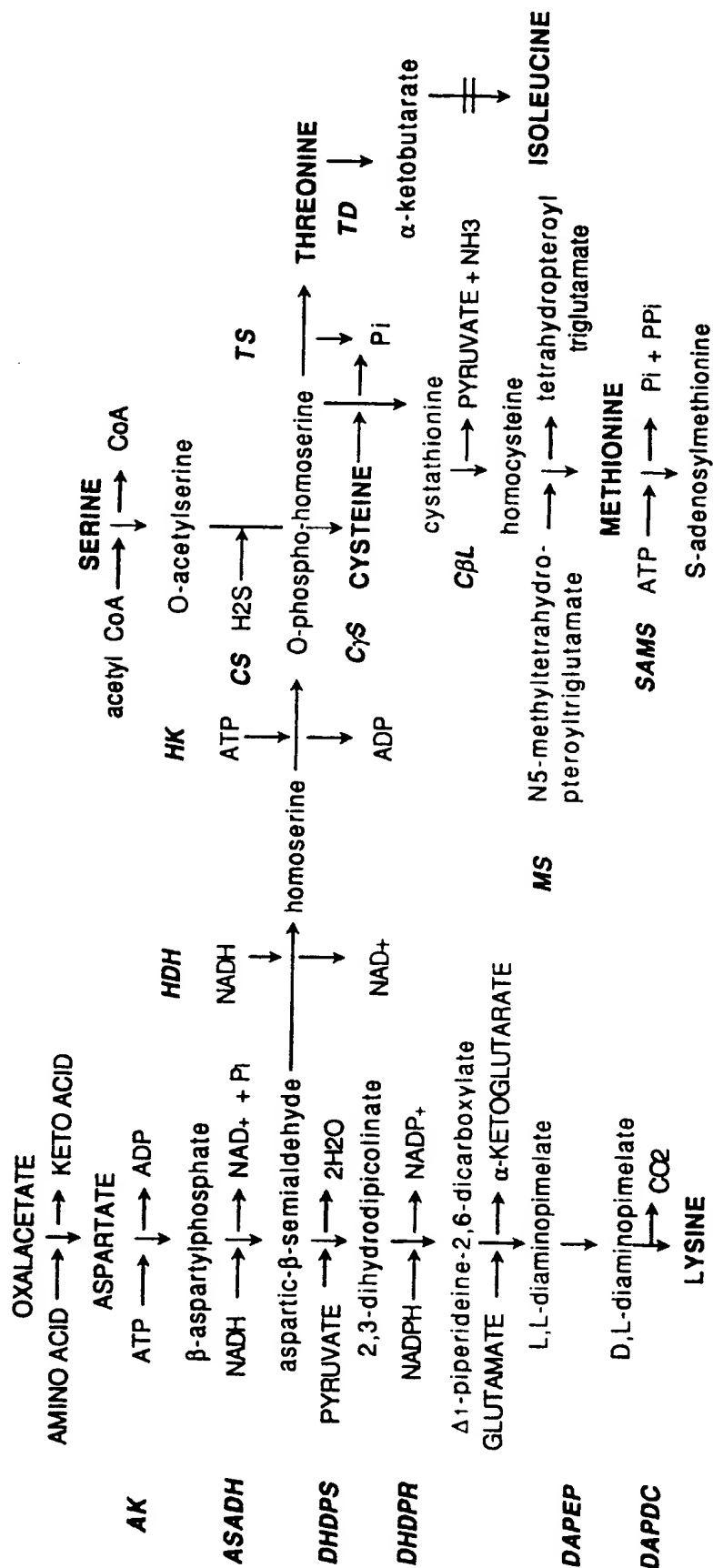
(d) treating the biosynthetic enzyme with a compound to be tested; and

(e) comparing the activity of the biosynthetic enzyme that has been treated with a test compound to the activity of an untreated biosynthetic enzyme,

25 thereby selecting compounds with potential for inhibitory activity.

1/18

FIG. 1



2/18

## FIG. 2

|     |              |                                                               |     |
|-----|--------------|---------------------------------------------------------------|-----|
| 1   | SEQ ID NO: 4 | .....                                                         | 60  |
|     | SEQ ID NO: 2 | .....KIGRRNAA...                                              |     |
|     | SEQ ID NO: 5 | MANQDLIPVVVNGAAGKMGREVIKAVAQAPDLQLVGAVDHNPSLQGGDIGEVVGIAPLEV  |     |
| 61  | SEQ ID NO: 4 | .....KVLCSQMPPSQSTI.....                                      | 120 |
|     | SEQ ID NO: 2 | PVLNDLTMVLGSIASRATGVVDFSEPSAVYDNVKQAAAFGLSSVVYPKIELETVTEL     |     |
|     | SEQ ID NO: 5 | PVLADLQSVLVLATQEKIQGMVDFTHPSGVYDNVRSIAIAYGVRPVVGTGTLSEQQIQDL  |     |
| 121 | SEQ ID NO: 4 | .....S.QCI...GLDAGEI.....                                     | 180 |
|     | SEQ ID NO: 2 | SAFCEKAS.GCLVAPTLSIGSVLLQQAIAQASFHYSNVEIVESRPNP.SDLPQDRAIQIA  |     |
|     | SEQ ID NO: 5 | GDFAEKASTGCLIAPNFAIGVLLMQQAQVQACQYFDHVEIIEELHHNQKADAPSGTAIKTA |     |
| 181 | SEQ ID NO: 4 | ..LNDLTMV.....LGSIAQTRA.....                                  | 240 |
|     | SEQ ID NO: 2 | NNISDLGQIYNR...EDMDSSSPARGQLLGEDGVRVHSMVLPGLVSSTSINFSGPGEMYT  |     |
|     | SEQ ID NO: 5 | QMLAEMGKTENPPAVEEKETIAGAKGGL.GPGQIPHSIRLPGLIAHQEVLFSGPGQLYT   |     |
| 241 | SEQ ID NO: 4 | NVKQA.....                                                    | 276 |
|     | SEQ ID NO: 2 | LRHDVANVQCLMPGLILAIRKWVRFKNLIYGLEKFL                          |     |
|     | SEQ ID NO: 5 | IRHDTTDRACYMPGVLGIRKVVVELKGLVYGLEKLL                          |     |

3/18

## FIG. 3

```
1      60
SEQ ID NO: 7 L.....
SEQ ID NO: 9 VS.....
SEQ ID NO: 11 MAITATISVPLTSPSRRTLTSVNSLSPLSTRSTLPTPQRTFKYPNSRLVSSMSTETAVK
SEQ ID NO: 13 .....
SEQ ID NO: 14 .....

61      120
SEQ ID NO: 7 .....
SEQ ID NO: 9 .....
SEQ ID NO: 11 TSSASFLNRKESGFLHFAKYHGLGNDLFVLDNRDSEPKISAQKAVQLCDRNFVGADGV
SEQ ID NO: 13 .....ALHFVKYQGLGNDLFIMVDNRDSAVPKVTPEEAQKLCDRNFVGADGV
SEQ ID NO: 14 .....MALSFESKYHGLGNDLFILVDNRQSTPCLTPDQAQQLCDRHFVGADGV

121      180
SEQ ID NO: 7 .....PEMCGNGVRCFARFIAELENLQGTNRFTIHTGAGKIV
SEQ ID NO: 9 IFVMPGVNGADYTMRIFNSDGSPEMCGNGVRCFARFIAELENLQGTSHFKIHTGAGLI
SEQ ID NO: 11 IFVLPGISGTDYTMRIFNSDGSPEMCGNGVRCFAKFSQLENLHGRHSETIHTGAGLI
SEQ ID NO: 13 IFVLPGVNGADYTMRIFNSDGSNRNVWX.GFV.....
SEQ ID NO: 14 IFALPGQGGTDYTMRIFNSDGSPEMCGNGIRCLAKFLADLEGVEEK.TYRIHTLAGVIT

181      240
SEQ ID NO: 7 PEIQSDGQVKVDMGEPILSGLDIPTKLLATKNKAVVQAEALAVEGLTWHVTCVSMGNPHCV
SEQ ID NO: 9 PEIQNDGKVKVDMGQPIIAC.....
SEQ ID NO: 11 PEVLEDGNVRVDMGEPVLKALDVPTKLPANKDNAVVKSQLVVDGVIWHVTCVSMGNPHCV
SEQ ID NO: 13 .....
SEQ ID NO: 14 POLLADGQVKVDMGEPQLLAELIPTTLAPAGEK.VVDLPLAVAGQTWAVTCVSMGNPHCL
```

4/18

## FIG.3 (Continued)

|               |     |                                   |     |                      |              |
|---------------|-----|-----------------------------------|-----|----------------------|--------------|
| SEQ ID NO: 7  | 241 | TFGANELKVLQVDDLLKSEIGPKFEHHEMF    | 300 | TFTVQVLSRSHLKMRVW    | GAGATL       |
| SEQ ID NO: 9  |     | .....                             |     | .....                |              |
| SEQ ID NO: 11 |     | TFSREGSQNLLVDELKLA                |     | TFTVQVLSNHLKMRVW     | GAGATL       |
| SEQ ID NO: 13 |     | .....                             |     | .....                |              |
| SEQ ID NO: 14 |     | TFVDD.....VDSLNLTEIGPLFEHHPQFSQRT |     | TFIQVLGSDRLKMRVW     | GAGITL       |
|               |     |                                   |     |                      |              |
| SEQ ID NO: 7  | 301 | ACGTGACAVVAAVLEGRAERKCVVDLP       | 359 | PGGPLEIEWREDDNHVYMTG | PAEVVFGSVVH  |
| SEQ ID NO: 9  |     | .....                             |     | .....                |              |
| SEQ ID NO: 11 |     | ACGTGACATVAAVLEGRAGRNCTVDLP       |     | GGPLQIEWREEDNHVYMTG  | SADVVYGS     |
| SEQ ID NO: 13 |     | .....                             |     | .....                |              |
| SEQ ID NO: 14 |     | ACGTGACATVAAVLTGRDRRCTVELP        |     | GGNLEIEWSAQDNRLYMTG  | PAQRFVSGQAEI |



5/18

## FIG. 4

|               |     |                                                                |     |                    |
|---------------|-----|----------------------------------------------------------------|-----|--------------------|
| SEQ ID NO: 24 | 1   | ASSSLFQSLPESLQTSK.PYAPPKPAAHFV'VRA.....                        | 50  | QSPLTQNNSSSKHRRPAD |
| SEQ ID NO: 25 |     | .....                                                          |     | .....              |
| SEQ ID NO: 27 |     | LSSCLFNASVSSLNPKQDPIRRHRSTSLRHRPVVISCTADGNNIKAPIETAVKPPHRT     |     | .....              |
|               |     | .....                                                          |     | .....              |
| SEQ ID NO: 20 | 61  | .....                                                          | 120 | MENGAATNGASEKSHSPS |
| SEQ ID NO: 22 |     | .....                                                          |     | .....              |
| SEQ ID NO: 24 |     | ENIRDEARRINAPHDHLFSAKYVPFNADSSSSSTESYSLSLDEIVYRSQSGGLLDVQHDM   |     | .....              |
| SEQ ID NO: 26 |     | .....                                                          |     | .....              |
| SEQ ID NO: 27 |     | DNIRDEARR.NRSNAVNPFSAKYVPFNA...APGSTESYSLSLDEIVYRSRSGGLLDVEHDM |     | .....              |
|               |     | .....                                                          |     | .....              |
| SEQ ID NO: 16 | 121 | .....                                                          | 180 | .....              |
| SEQ ID NO: 13 |     | .....                                                          |     | .....              |
| SEQ ID NO: 20 |     | QTYLSTRGDDIGLSFETVV...                                         |     | .....              |
| SEQ ID NO: 22 |     | .....                                                          |     | .....              |
| SEQ ID NO: 24 |     | DALKRFEDGEYWRNLFDSRVGKTTWPYGSVWSKKEWLPEIHDDDIVSAFEGNSNLFWAE    |     | .....              |
| SEQ ID NO: 26 |     | .....                                                          |     | .....              |
| SEQ ID NO: 27 |     | EALKRFDGAYWRDLFDSRVGKSTWPYGSVWSKKEWLPEIDDDDIVSAFEGNSNLFWAE     |     | .....              |
|               |     | .....                                                          |     | .....              |
| SEQ ID NO: 16 | 181 | .....                                                          | 240 | VGCASTGDTSA        |
| SEQ ID NO: 18 |     | .....                                                          |     | .....              |
| SEQ ID NO: 20 |     | LKGLAADGGFLPEEVPAAATEWQSWKDLPYTELAVKV.....                     |     | .....              |
| SEQ ID NO: 22 |     | .....                                                          |     | .....              |
| SEQ ID NO: 24 |     | REGKQFLGMNDLWVKHCGISHTGSFKDLGMTVLVSQVNRRLRKMNPVVGVCASGTGDTSA   |     | .....              |
| SEQ ID NO: 26 |     | .....                                                          |     | .....              |
| SEQ ID NO: 27 |     | REGKQFLGMNDLWVKHGGISHTGSFKDLGMTVLVSQVNRRLRKMNPVVGVCASGTGDTSA   |     | .....              |

6/18

## FIG. 4 (Continued)

|              |                                                               |
|--------------|---------------------------------------------------------------|
| 241          | 300                                                           |
| SEQ ID NO:16 | ALSAACAAAGIPAIVFLPADRISLQOLIQPIANGATVLSLDTDFDGCMLIREVTAELPI   |
| SEQ ID NO:18 | .....                                                         |
| SEQ ID NO:20 | .LSLYISPAEVPTE.....DLRALVER.....                              |
| SEQ ID NO:22 | .....                                                         |
| SEQ ID NO:24 | ALSAACASAAIPSIIVFLPANKISLAQLVQPIANGAFVLSIDTDFDGCMLIREVTAELPI  |
| SEQ ID NO:26 | .....LIQPIANGATVLSLDTDFDGCMLIREVTAELPI                        |
| SEQ ID NO:27 | ALSAACASAGIPSIIVFLPANKISMAQLVQPIANGAFVLSIDTDFDGCMLIREITAEPLI  |
| 301          | 360                                                           |
| SEQ ID NO:16 | YLANSLNPL.RLEGQKTAIEILQFNWQVPDWVIPGGNLGNIYAFYKGFEMCRVLGLV     |
| SEQ ID NO:18 | .....                                                         |
| SEQ ID NO:20 | .....SYSTERSKEVVPLVKLEDNLHLELFHGPNYSE.....                    |
| SEQ ID NO:22 | .....                                                         |
| SEQ ID NO:24 | YLANSLNSL.KLEGQKTAIEILQFDWQVPDWVIPGNSLGNIAFYKGFKMFQELGLV      |
| SEQ ID NO:26 | YLANSLNSL.XLEGQKTAIRDIATXNWQVPGGLHIPRRQSXTFYAFLQGF.....       |
| SEQ ID NO:27 | YLANSLNSL.RLEGQKTAIEILQFDWQVPDWVIPGGNLGNIYAFYKGFKMCQELGLV     |
| 361          | 420                                                           |
| SEQ ID NO:16 | DRVPRLVCAQAANANPLYRYKYKSGWTEFEPQTAETTFASAIQIGDPVSVDRAVVALKATD |
| SEQ ID NO:18 | .....                                                         |
| SEQ ID NO:20 | .....KDCALQFLGNLXEYF.....                                     |
| SEQ ID NO:22 | .....                                                         |
| SEQ ID NO:24 | DKIPRLVCAQAANADPLYLYFKSGWKEFKPVKSTTFASAIQIGDPVSIDRAVHALKSCD   |
| SEQ ID NO:26 | .....                                                         |
| SEQ ID NO:27 | DRIPRMVCAQAANANPLYLHYKSGWKDEKPMTASTTFASAIQIGDPVSIDRAVYALKKCN  |
| 421          | 480                                                           |
| SEQ ID NO:16 | GIVEEATEEELMDATALADRTGMFACPHGTGVALAALFKLQQRRIIGPNDRTVVVSTAHL  |
| SEQ ID NO:18 | .....                                                         |
| SEQ ID NO:20 | .....                                                         |
| SEQ ID NO:22 | .....DAMVQADSTGMFICPHTGTVALAALIKLRNMGVIGAGERVVVVSTAHL         |
| SEQ ID NO:24 | GIVEEATEEELMDATAQADSTGMFICPHTGTVALTALFKLRNSGVIKATDRTVVVSTAHL  |
| SEQ ID NO:26 | .....                                                         |

7/18

## FIG. 4 (Continued)

|               |                                                                 |
|---------------|-----------------------------------------------------------------|
| SEQ ID NO: 27 | GIVEEATEEEELMDAMAQADSTGMFICPHTGTGVALTALFKLRNQGVIAPTDRTVVYSTAHGL |
|               | 481                                                             |
| SEQ ID NO: 16 | KFTQSKIDYHDKNIKDMVCQYANPPISVKADFGSVMVDVLQKN                     |
| SEQ ID NO: 18 | .....LNGKI...                                                   |
| SEQ ID NO: 20 | .....MACKYSNPPVSVKADFGAVMDVLKKR.....LKGKL...                    |
| SEQ ID NO: 22 | KFAQSKIDYHSGGLIPGMG.RYANPLVSVKADFGSVMVDVLKDSCTTSPPTLTSLDVAK     |
| SEQ ID NO: 24 | KFTQSKIDYHSDIKDMACRYANPPMQVKADFGSVMVDVLKTY.....LQSKA..H         |
| SEQ ID NO: 26 | .....                                                           |
| SEQ ID NO: 27 | KFTQSKIDYHSNAIPDMACRFSNPPVDVKADFGAVMDVLKSY.....LGSNTLTLS        |

FIG. 5

[illegible]

9/18

## FIG. 5 (Continued)

|               |     |                                                                  |     |
|---------------|-----|------------------------------------------------------------------|-----|
| SEQ ID NO: 29 | 301 | GETVVAITSGANNFDRRLVTELADVGRKREAVLATFLPERQGSFKKFTLVGRMNITE        | 360 |
| SEQ ID NO: 31 |     | ..NIVAITSGANNFDRKLVRVVTETELANVGRKQEAFLATVMAEEPGSKQFCFCELVGQMNITE |     |
| SEQ ID NO: 33 |     | .....                                                            |     |
| SEQ ID NO: 34 |     | NQTLVAVTSGANNFDRMRFVAERAEVGEAREAVFAVTIPEERGSFKRFCSLVGDRNVTE      |     |
|               |     |                                                                  |     |
| SEQ ID NO: 29 | 361 | FKYRYDSNAKDALVLYSVGIYTDNELGAMMDRMESAKLRTVNLTDNDLAKDHLRYFIGGR     | 420 |
| SEQ ID NO: 31 |     | FKYRYNSNEK.AVVLYSVGVHTISELRAMQERMESSQLKTYNLTESDLVKDHLRYLMGGR     |     |
| SEQ ID NO: 33 |     | .....                                                            |     |
| SEQ ID NO: 34 |     | FNYRI.ADAQSAHIFVGVQIRRRGESADIAANFESHGFKTADLTHDELSKEHIRYMVGGR     |     |
|               |     |                                                                  |     |
| SEQ ID NO: 29 | 421 | SEIK.DELVYRFFPERPGALMKFLDTFSPRWNISLFHYRAQGEAGANVLVGIQVPPAEF      | 480 |
| SEQ ID NO: 31 |     | SNVQ.NEVFVVSXPRTGTALMKFLDXFSPRWDISL.....                         |     |
| SEQ ID NO: 33 |     | .....RPGALMKFLDPFSPRWNISLFHYRGEGETGANVLVGIQVPKSEM                |     |
| SEQ ID NO: 34 |     | SPLALDERLFRFEFFPERPGALMKFLSSMAPDWNISLFHYRNQGADYSSILVGLQVPQADH    |     |
|               |     |                                                                  |     |
| SEQ ID NO: 29 | 481 | DEFKSHANNLGYEYMSEHNNEIYRLLLRDPKV                                 | 512 |
| SEQ ID NO: 31 |     | .....                                                            |     |
| SEQ ID NO: 33 |     | DEFHDRANKLGIDYKVVVNDDDFQLLMH....                                 |     |
| SEQ ID NO: 34 |     | AEFERFLAALGYPYVEESANPAYRLFLS....                                 |     |

10/18

FIG. 6

|              |      |                                                                 |      |
|--------------|------|-----------------------------------------------------------------|------|
| SEQ ID NO:36 | 126  | GCAGATCAAGAGAGATGGCAGCTCTCGACACACCTTCTTACACCTCGGAGTGTGTAACG     | 185  |
|              |      |                                                                 |      |
| SEQ ID NO:37 | 774  | GCAGATAGGAGAGATGGCGCACTTGATACCTTCTTACCTCGGAGTGTGTAACG           | 833  |
| SEQ ID NO:36 | 186  | AGGGACACCCCTGACAAGCTCTGCGACCAAGTCTCAGATGCCGTTCTTGACCGTTGCCTTG   | 245  |
|              |      |                                                                 |      |
| SEQ ID NO:37 | 834  | AGGGCACCCCTGACAAGCTCTGCGACCAAGTCTCAGATGCTGTGCTTGATGCCCTGCCCTCG  | 893  |
| SEQ ID NO:36 | 246  | CTGAGGACCCCTGACAGCAAGTTGCTTGTGAGACCTGCACCAAGACCAACATGGTTCATGG   | 305  |
|              |      |                                                                 |      |
| SEQ ID NO:37 | 894  | CCGAGGACCCCTGACAGCAAGTTCGCTTGTGAGACCTGCACCAAGACCAACATGGTTCATGG  | 953  |
| SEQ ID NO:36 | 306  | TCCTTTGGTGAGATCACCAACAGGCCAATGTCGACTACGAGAGAGATTGTCAGGGAGACCT   | 365  |
|              |      |                                                                 |      |
| SEQ ID NO:37 | 954  | TCCTTTGGTGAGATCACCAACAGGCTAACGTTGACTATGAGAAGATTGTCAGGGAGACAT    | 1013 |
| SEQ ID NO:36 | 366  | GCCGCAACATTGGTTTTTGTGTCAAAACGATGTCGGGCTTGACGCTGACCACCTGCAAGGTGC | 425  |
|              |      |                                                                 |      |
| SEQ ID NO:37 | 1014 | GCCGTAACATCGGTTTTTGTGTCAAGTATGTCGGTCTCGATGCTGACCACCTGCAAGGTGC   | 1073 |
| SEQ ID NO:36 | 426  | TCGTGAACATTGAGCAGCAGTCCCCTGATATTGCTCAGGGTGTGCATGGCCACTTCACCA    | 485  |
|              |      |                                                                 |      |
| SEQ ID NO:37 | 1074 | TTGTGAACATCGAGCAGCAGTCCCCTGACATTGCACAGGGTGTGCACGGGCACTTCACCA    | 1133 |
| SEQ ID NO:36 | 486  | AGGCCCCGAGGAGATTGGAGCTGTCACCAAGGACACATGTTCCGGTATGCGACCCGATG     | 545  |
|              |      |                                                                 |      |
| SEQ ID NO:37 | 1134 | AGGCCCCTGAGGAGATTGGTGTGTCGACCAAGGACACATGTTTGGATATGCAACTGATG     | 1193 |
| SEQ ID NO:36 | 546  | AGACCCCTGAGTTGATGCCCCCTCAGCCATGTCCTTGCCACCAAGCTAGGTGCTCGTCTCA   | 605  |
|              |      |                                                                 |      |
| SEQ ID NO:37 | 1194 | AGACCCCTGAGTTGATGCCCCCTCAGCCATGTCCTTGCTACCAAGCTTGGCGCTCGTCTTA   | 1253 |









|              |      |                                                                |      |
|--------------|------|----------------------------------------------------------------|------|
| SEQ ID NO:38 | 560  | CCATGGTTGAGGCGCTGATGGGAAACCCAAAGTGACTGTTGAGTATTACAATGACACACGGT | 619  |
| SEQ ID NO:40 | 603  | GCCTGGTTGAGGCGCTGATGGCAAGACCCCAAGTTACTGTTGAGTATAGCAATGACAATGGT | 662  |
| SEQ ID NO:38 | 620  | GCCATGGTTCCAGTTGCGTGTCACACTGTGCTTATCTCCACCCCAACATGATGAGACTGTG  | 679  |
| SEQ ID NO:40 | 663  | GCCATGGTTCCAAATTAGGGTACACACTGTTCTTATCTCCACCCCAACATGAGACCGTT    | 722  |
| SEQ ID NO:38 | 680  | ACCAACGACGAAATTGCAGCTGACCTCAAGGAGCATGTGATCAAGCCGGTGATCCCGGAG   | 739  |
| SEQ ID NO:40 | 723  | ACCAATGATGAGATTGCCCGCGACCTTAAGGAGCATGTCATCAAAACCATCATCCACAG    | 782  |
| SEQ ID NO:38 | 740  | AAGTACCTTGATGAGAAAGACCAATTTCCACTTGAACCCCTCTGGCCGTTTGTGTCATTGGA | 799  |
| SEQ ID NO:40 | 783  | AAGTACCTTGATGAGAAATACTATTTCCACCTTAACCCATCTGGCCGATTCTGTTATTGGT  | 842  |
| SEQ ID NO:38 | 800  | GGTCCTCACGGTGATGCTGGTCTCACCGGCCGCAAGATCATCATCTTACGGAGGA        | 859  |
| SEQ ID NO:40 | 843  | GGACCTCATGGTGATGCTGGTCTCACTGGTCGTAATAATCATCATCGACACTTATGGTGGT  | 902  |
| SEQ ID NO:38 | 860  | TGGGGTGCTCATGGTGGTGCTTCTCCGGGAAGGATCCCACCAAGTTGATAGGAGT        | 919  |
| SEQ ID NO:40 | 903  | TGGGGTGCTCATGGTGGTGCTTCTCGGGCAAGACCCCAACCAAGGTCGACAGGAGT       | 962  |
| SEQ ID NO:38 | 920  | GGTGCTTACATTGTGAGACAGGCTGCTAAGAGCATTTGTGSCAAGTGGACTAGCCAGAAAG  | 979  |
| SEQ ID NO:40 | 963  | GGTGCATACATTGTAAGGCAGGCTGCAAGAGTATCGTAGCTAGTGGACTTGTCTCGTAGA   | 1022 |
| SEQ ID NO:38 | 980  | TGCATTGTGCAAGTGTCTTATGCCATTGGTGTGCCGAGCCCTTGTCTGTCTTTGTTGAC    | 1039 |
| SEQ ID NO:40 | 1023 | TGCATCGTGCAGGTATCTTATGCCATCGGTGTGCTGAGCCATTGCTGTGATTCTGTTGAC   | 1082 |





FIG. 8 (Continued)

FIG. 8 (Continued)



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant (for all designated States except US): E.I. DU PONT  
DE NEMOURS AND COMPANY [US/US]; 1007 Market  
Street, Wilmington, DE 19898 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): FALCO, Saverio, Carl  
[US/US]; 1902 Millers Road, Arden, DE 19810 (US).  
ALLEN, Stephen, M. [US/US]; 12 Stanton Avenue, West  
Chester, PA 19382 (US). RAFALSKI, J., Antoni [US/US];  
2028 Longcome Drive, Wilmington, DE 19810 (US). HITZ,  
William, D. [US/US]; 404 Hillside Road, Wilmington,  
DE 19807 (US). KINNEY, Anthony, John [US/US]; 609  
Lore Avenue, Wilmington, DE 19809 (US). ABELL, Lynn,  
Marie [US/US]; 5 Laurel Court, Wilmington, DE 19808  
(US). THORPE, Catherine, Jane [GB/GB]; 120 Ross Street,  
Cambridge CB1 3BU (GB).

(74) Agent: MAJARIAN, William, R.; E.I. du Pont de Nemours  
and Company, Legal Patent Records Center, 1007 Market  
Street, Wilmington, DE 19898 (US).

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LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY,  
KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH,  
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ML, MR, NE, SN, TD, TG).

## Published

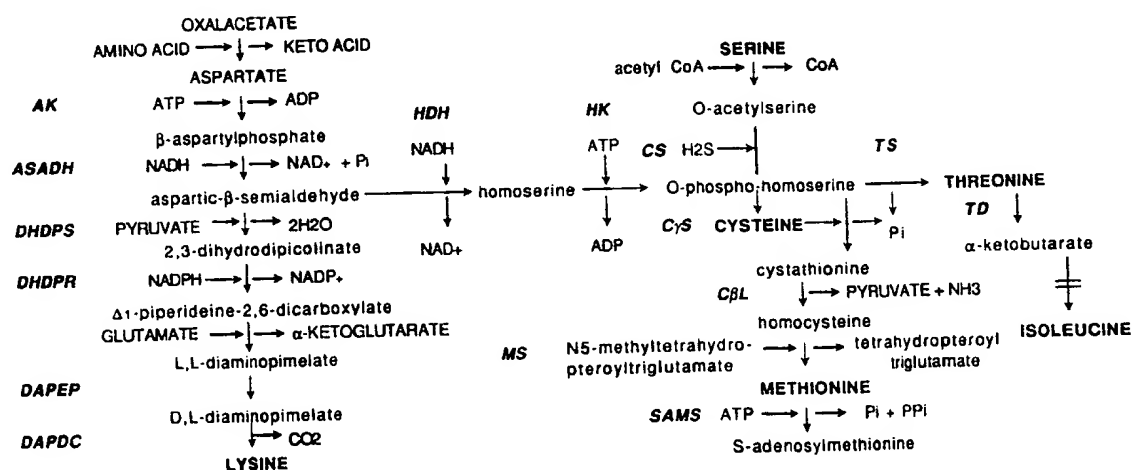
With international search report.

Before the expiration of the time limit for amending the claims  
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(88) Date of publication of the international search report:

4 March 1999 (04.03.99)

(54) Title: PLANT AMINO ACID BIOSYNTHETIC ENZYMES



## (57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a plant enzyme that catalyzes steps in the biosynthesis of lysine, threonine, methionine, cysteine and isoleucine from aspartate, the enzyme a member selected from the group consisting of: dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the enzyme in a transformed host cell.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 98/11692

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N9/02 C12N9/88 C12N9/90 C12N9/10  
C12Q1/68 G01N33/50 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q G01N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                           | Relevant to claim No. |
|------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X          | FENG, J. ET AL.: "unpublished"<br>EMBL SEQUENCE DATA LIBRARY, 10 May 1997,<br>XP002078204<br>heidelberg, germany<br>Accession No.B10032<br>---                                                                                                                                                                                               | 1                     |
| A          | SAITO, K., ET AL.: "modulation of<br>cysteine biosynthesis in chloroplasts of<br>transgenic tobacco overexpressing cysteine<br>synthase (O-Acetylserine(thiol)-lyase)"<br>PLANT PHYSIOLOGY,<br>vol. 106, 1994, pages 887-895, XP002078205<br>abstract, page 887, right column; page<br>888, left column; page 890-894; Fig. 10<br>---<br>-/- | 1-5,<br>50-55         |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

22 September 1998

Date of mailing of the international search report

15. 01. 99

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Holtorf, S

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/11692

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                    | Relevant to claim No. |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A          | YOUSSEFIAN, S., ET AL.: "tobacco plants transformed with the O-acetylserine (thiol) lyase gene of wheat are resistant to toxic levels of hydrogen sulphide gas" THE PLANT JOURNAL, vol. 4, no. 5, 1993, pages 759-769, XP002078206 abstract; Fig.1,2,3,5; page 760, left column, last paragraph; pages 764-767<br>--- | 1-5,<br>50-55         |
| A          | BOERJAN, W., ET AL.: "distinct phenotypes generated by overexpression and suppression of S-adenosyl-L-methionine synthetase reveal developmental patterns of gene silencing in tobacco" THE PLANT CELL, vol. 6, October 1994, pages 1401-1414, XP002078207 see the whole document<br>---                              | 1-5,<br>50-55         |
| A          | US 5 545 545 A (GENGENBACH BURLE G ET AL) 13 August 1996 Fig.1,2; Columns 1,3-7; examples 1,3,5,11<br>---                                                                                                                                                                                                             | 1-5,<br>50-55         |
| A          | US 5 451 516 A (MATTHEWS BENJAMIN F ET AL) 19 September 1995 see the whole document<br>---                                                                                                                                                                                                                            | 1-5,<br>50-55         |
| A          | WO 96 01905 A (DU PONT ;FALCO SAVERIO CARL (US)) 25 January 1996 pages 1,2,4,7; examples<br>---                                                                                                                                                                                                                       | 1-5,<br>50-55         |
| A          | WO 96 38574 A (PIONEER HI BRED INT) 5 December 1996 page 4,6,7,8,9; examples, claims<br>---                                                                                                                                                                                                                           | 1-5,<br>50-55         |
| A          | EP 0 485 970 A (YEDA RES & DEV) 20 May 1992 abstract, column 1-10; examples<br>---                                                                                                                                                                                                                                    | 1-5,<br>50-55         |
| A          | WO 97 07665 A (UNIV HAWAII) 6 March 1997 see the whole document<br>---                                                                                                                                                                                                                                                | 1-5,<br>50-55         |
| A          | CURIEN, G., ET AL.: "characterization of an arabidopsis thaliana cDNA encoding an S-adenosylmethionine sensitive threonine synthase" EMBL SEQUENCE DATA LIBRARY, 26 July 1996, XP002078253 heidelberg, germany cited in the application Accession No. L41666<br>---                                                   | 1-5,<br>50-55         |
|            | -/-                                                                                                                                                                                                                                                                                                                   |                       |

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/11692

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                     | Relevant to claim No. |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A        | <p>ESPARTERO, J., ET AL.: "differential accumulation of S-adenosylmethionine synthetase transcripts in response to salt stress"</p> <p>EMBL SEQUENCE DATA LIBRARY,<br/>23 November 1993, XP002078254<br/>heidelberg, germany<br/>cited in the application<br/>Accession No. Z24741</p> | <p>1-5,<br/>50-55</p> |
| A        | <p>---<br/>SCHWARTZ, D.H., ET AL.: "untitled"</p> <p>EMBL SEQUENCE DATA LIBRARY, 8 June 1996,<br/>XP002078255<br/>heidelberg, germany<br/>cited in the application<br/>Accession No. U49630<br/>-----</p>                                                                              | <p>1-5,<br/>50-55</p> |

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 98/11692

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-5 completely, 50-55 partially

#### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: claims 1-5 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Dihydropicolinate Reductase (DHDPR) from corn and rice; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

2. Claims: claims 6-10 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Diaminopimelate Epimerase (DAPEC) from corn, wheat, rice and soybean; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

3. Claims: Claims 11-15 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Synthase (TS) from corn; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

4. Claims: Claims 16-20 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Synthase (TS) from rice; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

5. Claims: Claims 21-25 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Synthase (TS) from soybean; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

6. Claims: Claims 26-30 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Synthase (TS) from wheat; recombinant expression of said genes in a transformed host cell; further methods to alter

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

7. Claims: Claims 31-35 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Deaminase (TD) from corn; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

8. Claims: Claims 36-40 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Deaminase (TD) from soybean; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

9. Claims: Claims 41-43 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for S-Adenosylmethionine Synthetase (SAMS) from corn; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

10. Claims: Claims 44-46 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for S-Adenosylmethionine Synthetase (SAMS) from soybean; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

11. Claims: Claims 47-49 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for S-Adenosylmethionine Synthetase (SAMS) from wheat; recombinant expression of said genes in a transformed host; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/11692

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s)                                                 | Publication<br>date                                                |
|-------------------------------------------|---|---------------------|----------------------------------------------------------------------------|--------------------------------------------------------------------|
| US 5545545                                | A | 13-08-1996          | NONE                                                                       |                                                                    |
| US 5451516                                | A | 19-09-1995          | NONE                                                                       |                                                                    |
| WO 9601905                                | A | 25-01-1996          | AU 2963695 A<br>BR 9510174 A<br>CA 2192550 A<br>EP 0769061 A<br>HU 77112 A | 09-02-1996<br>04-11-1997<br>25-01-1996<br>23-04-1997<br>02-03-1998 |
| WO 9638574                                | A | 05-12-1996          | AU 5956196 A<br>CA 2222673 A<br>EP 0828845 A                               | 18-12-1996<br>05-12-1996<br>18-03-1998                             |
| EP 0485970                                | A | 20-05-1992          | US 5367110 A                                                               | 22-11-1994                                                         |
| WO 9707665                                | A | 06-03-1997          | AU 6899796 A<br>EP 0871356 A                                               | 19-03-1997<br>21-10-1998                                           |

